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2-{3-[4-(Alkylsulfinyl)phenyl]-1-benzofuran-5-yl}-5-methyl-1,3,4-oxadiazole Derivatives as Novel Inhibitors of Glycogen Synthase Kinase-3β with Good Brain Permeability†

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Glycogen synthase kinase 3β (GSK-3β) inhibition is expected to be a promising therapeutic approach for treating Alzheimer's disease. Previously we reported a series of 1,3,4-oxadiazole derivatives as potent and highly selective GSK-3β inhibitors, however, the representative compounds **1a**,**b** showed poor pharmacokinetic profiles. Efforts were made to address this issue by reducing molecular weight and lipophilicity, leading to the identification of oxadiazole derivatives containing a sulfinyl group, (*S*)-**9b** and (*S*)-**9c**. These compounds exhibited not only highly selective and potent inhibitory activity against GSK-3β but also showed good pharmacokinetic profiles including favorable BBB penetration. In addition, (*S*)-**9b** and (*S*)-**9c** given orally to mice significantly inhibited cold water stress-induced tau hyperphosphorylation in mouse brain.

Introduction

Alzheimer's disease (AD[a]) is a dementia of middle- to old-aged individuals and was discovered in 1906 by Alois Alzheimer. AD has pathological features of neurofibrillary tangles (NFT), senile plaque, and neuronal death. Neurofibrillary degeneration appears to be required for the clinical expression of the disease, and formation of NFT and neuronal death correlate with the presence and or the degree of dementia in AD.[1] Thus, inhibition of NFT formation is a viable strategy for the treatment of AD. NFT is composed of the abnormally hyperphosphorylated microtubule-associated protein tau. Hyperphosphorylation of tau protein causes destabilization of microtubules, which then aggregate to form NFTs. Normally two to three residues on tau bear phosphate, however, hyperphosphorylated tau protein in AD and other tauopathies have close to nine phosphates.[2] Although phosphorylation of tau protein is regulated with balance in the activity levels of kinases and phosphatases, hyperphosphorylation of tau is thought to be induced mainly by the serine/threonine kinase GSK-3β.[3] Overexpression of GSK-3β in cultured cells and in transgenic mice results in hyperphosphorylation of tau at several of the same sites seen in AD and inhibition by lithium chloride attenuates phosphorylation in these models.[4−11] These studies suggest that GSK-3β is associated with AD progression, and GSK-3β inhibitors are expected to be promising therapeutic agents for AD. Many GSK-3β inhibitors that are ATP competitive inhibitors have been reported and their medicinal use has been reviewed in the literature.[12,13] Maleimide derivatives have been reported by many groups.[14,15] A variety of compounds with different chemical structures have been reported, such as 2,5-diaminopyrimidine,[16] 1-aza-9-fluorene,[17] and thiazolo[5,4-*f*]quinazoline derivatives.[18] In addition, natural product derived GSK-3β inhibitors such as indirubins,[19] paullones,[20] and manzamines[21] have been described. In this paper, we report the design and synthesis of novel small molecular GSK-3β inhibitors having a 1,3,4-oxadiazole scaffold and the evaluation of their in vivo efficacy against tau hyperphosphorylation in mice. Previously, we reported a series of novel 1,3,4-oxadiazole derivatives as potent and highly selective GSK-3β inhibitors. Among them, 2-(1-benzofuran-5-yl)-1,3,4-oxadiazole derivative **1a** and 2-(1*H*-benzimidazol-6-yl)-1,3,4-oxadiazole derivative **1b** exhibited high potency with IC_{50} values of 3.5 and 2.3 nM, respectively (Figure 1).[22]

Although the X-ray cocrystal structure of compound **1b** with GSK-3β was not fully elucidated due to cleavage of the

†PDB codes for GSK-3β complexes **1b** and (*S*)-**9b** are 3F88 and 3GB2, respectively.

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[a] Abbreviations: Ac, acetyl; AcOH, acetic acid; AD, Alzheimer's disease; AIBN, 2,2′-azobis(2-methylpropionitrile) BBB, blood−brain barrier; *n*-BuOH, *n*-butanol; $CDCl_3$, deuterated chloroform; CDI, 1,1′-carbonyldiimidazole; CWS; cold water stress; DBU, 1,8-diazabicyclo-[5.4.0]undec-7-ene; DMA, *N*,*N*-dimethylacetamide; DMB, 2,4-dimethoxybenzyl; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; DMSO-d_6, dimethyl sulfoxide-d_6; EDC, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride; ERBB, v-erb-a erythroblastic leukemia viral oncogene homologue; EtOAc, ethyl acetate; EtOH, ethanol; FGFR, fibroblast growth factor receptor; GSK-3β, glycogen synthase kinase 3β; HOBt, 1-hydroxybenzotriazole; HPLC, high-performance liquid chromatography; IMAC, immobilized metal-chelate affinity chromatography; *m*-CPBA, *m*-chloroperbenzoic acid; MeOH, methanol; NBS, *N*-bromosuccinimide; NEt_3, triethylamine; NFT, neurofibrillary tangles; NIS, *N*-iodosuccinimide; PDGFR, platelet-derived growth factor receptor; PhCl, chlorobenzene; RIPA, radioimmunoprecipitation assay; rt, room temperature; TFA, trifluoroacetic acid; Tf_2NPh, *N*-phenyl-bis(trifluoromethanesulfonimide); THF, tetrahydrofuran; TsCl, *p*-toluenesulfonyl chloride; *p*-TsOH, *p*-toluenesulfonic acid monohydrate; VEGFR, vascular endothelial growth factor receptor.

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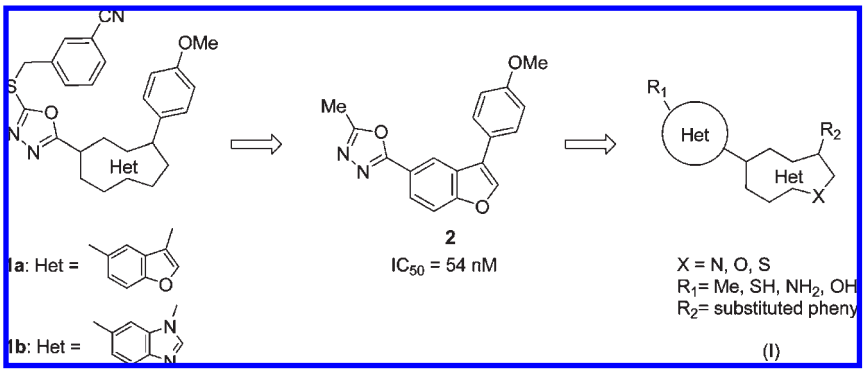


Figure 1. Design of compound (**I**) from compound **2**.

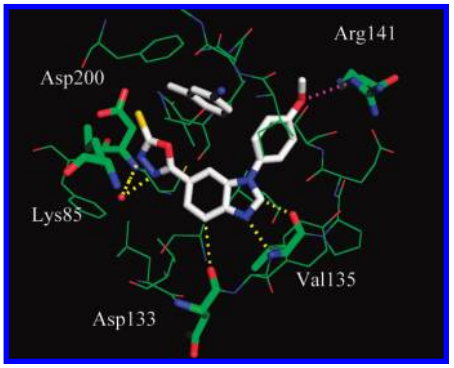


Figure 2. X-ray cocrystal structure of **1b** in complex with GSK-3β. The figure was prepared with PyMOL.[23]

S−C bond in the X-ray beam to form the debenzylated compound, some information could be gathered from the partial structure solution. The structure (Figure 2) shows the following major hydrogen bond interactions: the benzimidazole ring and the hinge region of GSK-3β, N3-nitrogen of the oxadiazole ring and NH of Asp200, the N4-nitrogen of the oxadiazole ring and a conserved water molecule, and the 4-methoxy group of the phenyl ring and Arg141.

Unfortunately, the bioavailability of compounds **1a** and **1b** in rat was poor, probably due to poor solubility and instability in rat liver microsomes. To improve the solubility and metabolic stability of these derivatives, we focused on modification of the highly lipophilic and metabolically labile S-benzyl group, replacing it with a methyl group. The methyl oxadiazole derivative **2** was found to retain potent inhibitory activity (IC_{50} = 54 nM), while compound **2** still showed poor metabolic stability and bioavailability. However, the low molecular weight (MW 306.3) of compound **2** made us consider it worthwhile to perform further optimization and SAR studies on this series of compounds. Thus, we designed a series of compounds based on this scaffold (**I**). On the basis of the X-ray cocrystal structure of **1b** and GSK-3β, we particularly explored other polar substituents on the phenyl ring in place of the methoxy group interacting with Arg141 in order to improve metabolic stability and solubility and also modified the hinge binding portion and the oxadiazole moiety.

Chemistry

Commercially available 2,3-dihydro-1-benzofuran-5-carbaldehyde **3** was directly converted to ester **4** by using iodine and KOH. The resulting ester **4** was treated with *N*-bromosuccinimide (NBS), followed by bromination with bromine afforded 3-bromobenzofuran **5**. Hydrazide **6** was prepared by the addition of hydrazine to **5**. Reaction of the hydrazide **6** with triethyl orthoacetate afforded oxadiazole **7**. The 3-substituted benzofurans **8a**−**d**,**f**−**j**,**l**,**n** were prepared by reaction of **7** with corresponding aryl boronic acid. Hydrolysis of **8d** afforded carboxylic acid **8e**. Reduction of ketone **8j** with $NaBH_4$ gave alcohol **8k**. Reaction of phenol **8l** with *N*-phenyl-bis(trifluoromethanesulfonimide) ($PhNTf_2$) and subsequent Pd-coupling with dimethyl phosphite gave phosphate **8m**. Oxidation of methyl sulfides **8a**,**i** and ethyl sulfide **8b** with *m*-chloroperbenzoic acid (*m*-CPBA) gave sulfoxides **9a**−**c** and sulfone **9d** (Scheme 1).

Coupling of 4-(methylthio)phenylboronic acid with 3-bromobenzofuran intermediate **5** gave **10**, which was treated with hydrazine to afford hydrazide **11**. Hydroxy-oxadiazole **12a** was obtained from hydrazide **11** by the reaction with 1,1′-carbonyldiimidazole. Reaction of **11** with cyanogen bromide gave amino-oxadiazole **12b**. Sulfides **12a**,**b** were oxidized to sulfoxides **13a**,**b** with oxone or *m*-CPBA. Sulfide **10** was oxidized to sulfoxide **14**, then reacted with hydrazine, carbon disulfide, and triethylamine (NEt_3) followed by acidification to yield 1,3,4-oxadiazole-2-thiol **15** (Scheme 2).

Next, replacement of the benzofuran ring to other 6−5 fused heterocycles was performed. The syntheses are outlined in Schemes 3−5. Ester **16**[24] and 4-(methylthio)aniline were reacted in DMF to yield diphenyl amine **17**. The nitro group of **17** was reduced with sodium hydrosulfite to provide aniline **18**, which was cyclized with formic acid to form benzimidazole **19**. Reaction with hydrazine, followed by acetylation and cyclization, afforded oxadiazole **22**. Oxidation of **22** with *m*-CPBA gave sulfoxide **23** (Scheme 3).

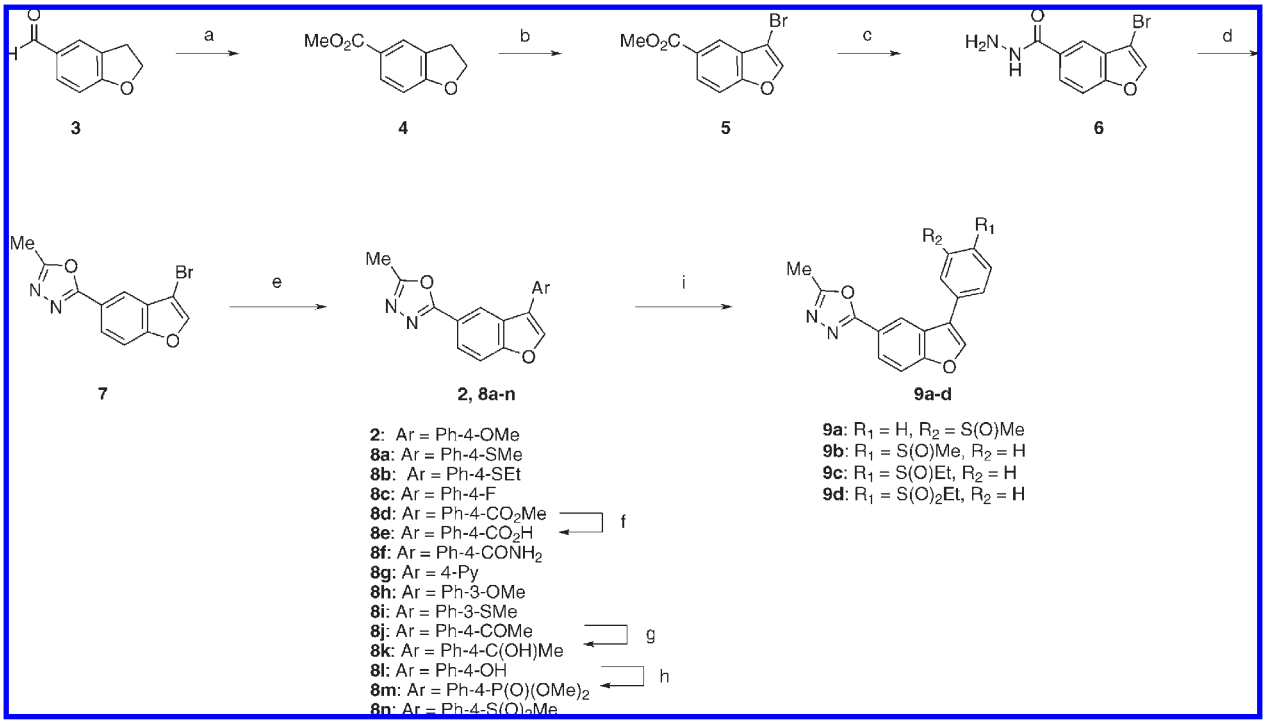
Esters **24**[25−28] were halogenated and coupled with [4-(methylthio)phenyl]boronic acid to provide **26**. Treatment with hydrazide and cyclization gave oxadiazoles **28**. Oxidation of sulfides **28** afforded sulfoxides **29** (Scheme 4).

Condensation of carboxylic acid **30**[29] with acetohydrazide and cyclization using *p*-toluenesulfonyl chloride (TsCl) provided oxadiazole **32**. Coupling and oxidation of the sulfide **33** afforded sulfoxide **34** (Scheme 5).

The preparation of 1,2,4-triazole **36**, 1,3,4-thiadiazole **39**, 1,2,4-oxadiazoles **41** and **46**, and pyridazine **51** were performed according to Schemes 6−8. Oxadiazole **9b** was reacted with 2,4-dimethoxybenzylamine, followed by cleavage of the 2,4-dimethoxybenzyl (DMB) group using trifluoroacetic acid (TFA) to give triazole **36**. Hydrazide **11** was condensed with acetic acid (AcOH) and cyclized upon treatment with Lawesson's reagent to give thiadiazole **38**, which was oxidized to provide sulfoxide **39** (Scheme 6).

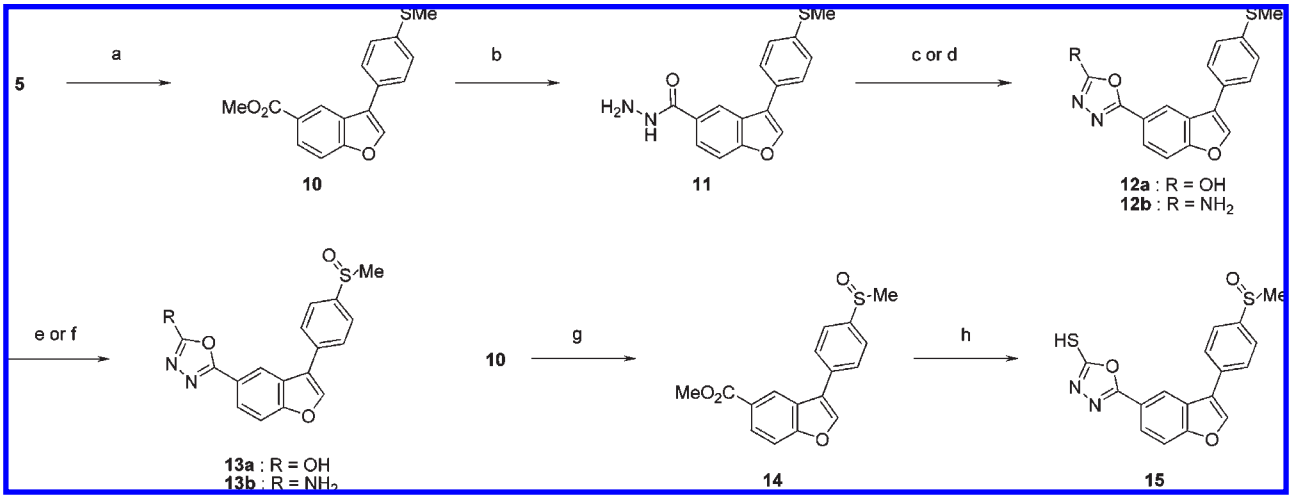
1,2,4-Oxadiazole **40** was synthesized from ester **10** and acetamidoxime and subsequent oxidation yielded sulfoxide **41**. Hydrolysis of **10** with NaOH followed by condensation

Scheme 1[a]



[a] Reagents and conditions: (a) I_2, KOH, MeOH, 0 °C, 86%; (b) (1) NBS, AIBN, PhCl, 80−85 °C, (2) Br_2, CH_2Cl_2, 0 °C∼rt, then KOH, MeOH-THF, 0 °C, 89%; (c) $NH_2NH_2 \cdot H_2O$, MeOH, reflux, 95%; (d) $CH_3C(OEt)_3$, 120 °C, 97%; (e) $Pd(PPh_3)_4$, Na_2CO_3, DME-H_2O, $ArB(OH)_2$, reflux, 42−95%; (f) 1 M NaOH, THF-MeOH, 60 °C, 83%; (g) $NaBH_4$, EtOH, 0 °C, 76%; (h) (1) $PhNTf_2$, NaH, THF, 92%, (2) $HP(O)(OMe)_2$, $Pd(PPh_3)_4$, *i*-Pr_2NEt, toluene, 100 °C, 55%; (i) *m*-CPBA, CH_2Cl_2, 0 °C or rt, 26−84%.

Scheme 2[a]



[a] Reagents and conditions: (a) 4-(methylthio)phenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, DME-H_2O, reflux, 85%; (b) $NH_2NH_2 \cdot H_2O$, MeOH, reflux, 95%; (c) CDI, Et_3N, DMF, 0 °C, 74% (**12a**); (d) BrCN, DMA, 79% (**12b**); (e) *m*-CPBA, CH_2Cl_2, 80% (**13a**); (f) oxone, THF-H_2O, 0 °C, 6% (**13b**); (g) *m*-CPBA, CH_2Cl_2, 93%; (h) (1) $NH_2NH_2 \cdot H_2O$, MeOH, reflux; (2) CS_2, NEt_3, EtOH, reflux, 47%.

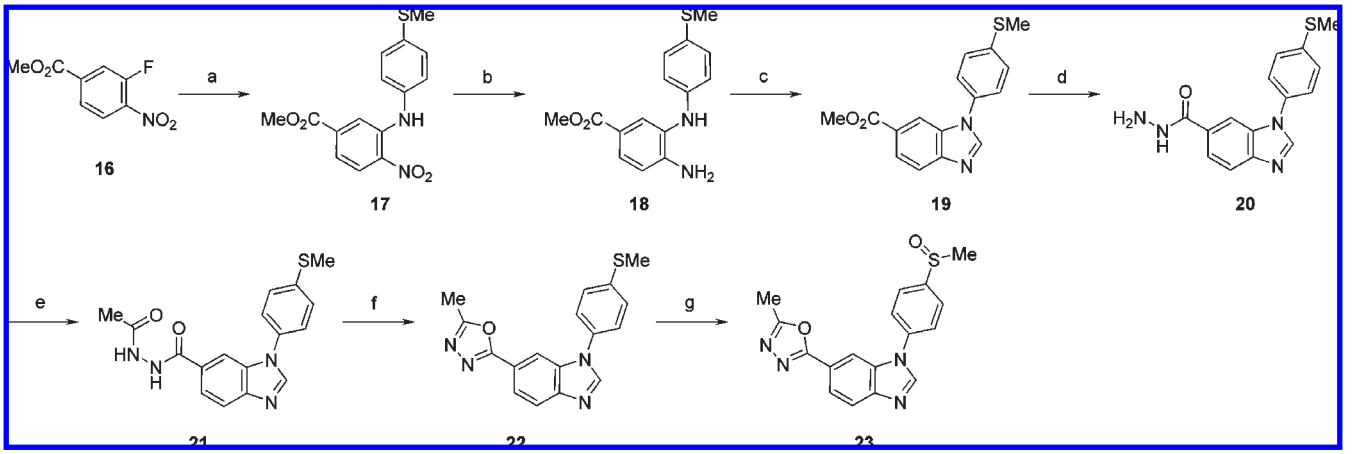
with ammonium hydroxide afforded amide **43**. Dehydration of **43** was accomplished with thionyl chloride to give nitrile **44**. Treatment of **44** with hydroxylamine hydrochloride and $NaHCO_3$ gave the amidoxime intermediate, which was acylated with acetic anhydride in dioxane to give oxadiazole **45**, followed by oxidation to yield sulfoxide **46** (Scheme 7).

Aldehyde **3** was converted to 3-bromobenzofuran **47**, which was condensed with methyl vinyl ketone to provide diketone **48**. Reaction of diketone **48** with hydrazine afforded pyridazine **49**, which was coupled with 4-(methylthio)phenylboronic acid and subsequent oxidation gave sulfoxide **51** (Scheme 8).

Results and Discussion

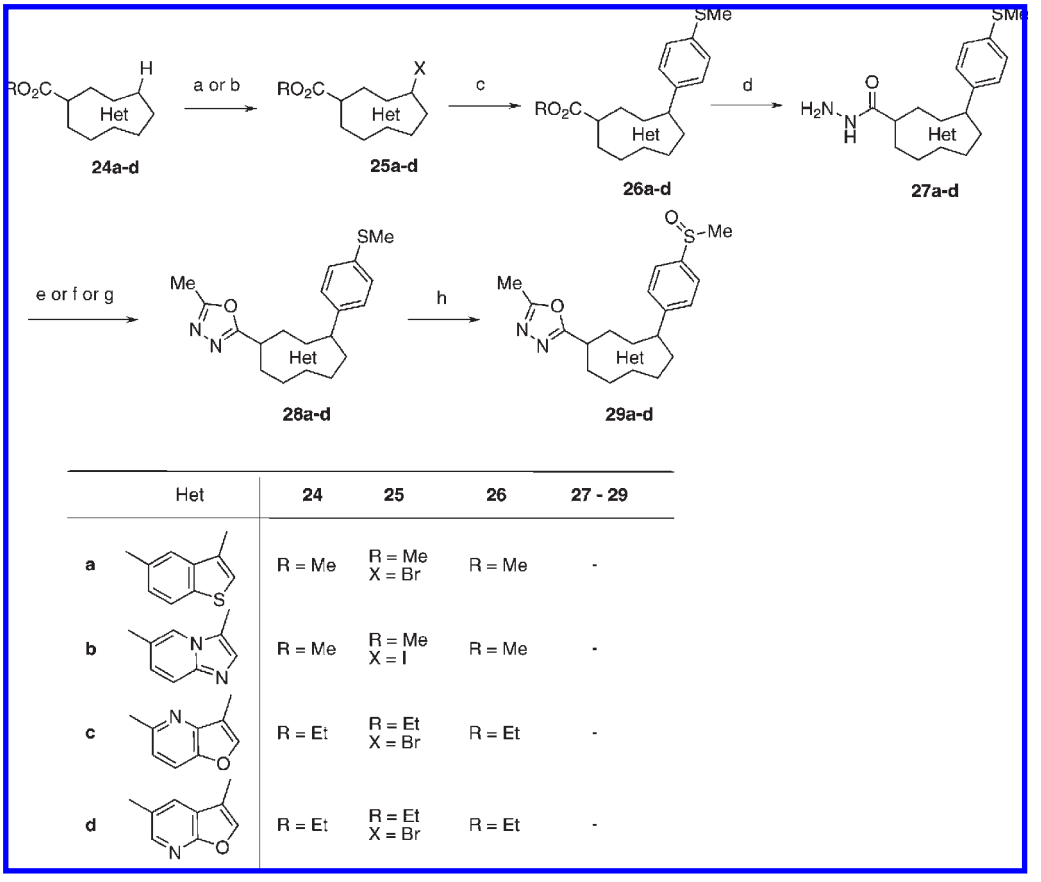
The compounds synthesized were evaluated for GSK-3β inhibitory activity in a non-RI kinase assay using Kinase-Glo reagents and the results are shown in Tables 1−5. First, we synthesized compounds with various substituents on the 3-phenyl ring (Table 1). Replacement of methoxy group of **2** with carboxylic acid or carboxamide (**8e**,**f**) showed similar

Scheme 3[a]



[a] Reagents and conditions: (a) 4-(methylthio)aniline, DMSO, 110 °C, 93%; (b) $Na_2S_2O_4$, THF-EtOH, 0 °C∼rt, 100%; (c) HCO_2H, 100 °C, 74%; (d) $NH_2NH_2 \cdot H_2O$, EtOH, 86%; (e) AcCl, DMA, 0 °C, 100%; (f) TsCl, pyridine, 90 °C, 66%; (g) *m*-CPBA, CH_2Cl_2, 85%.

Scheme 4[a]

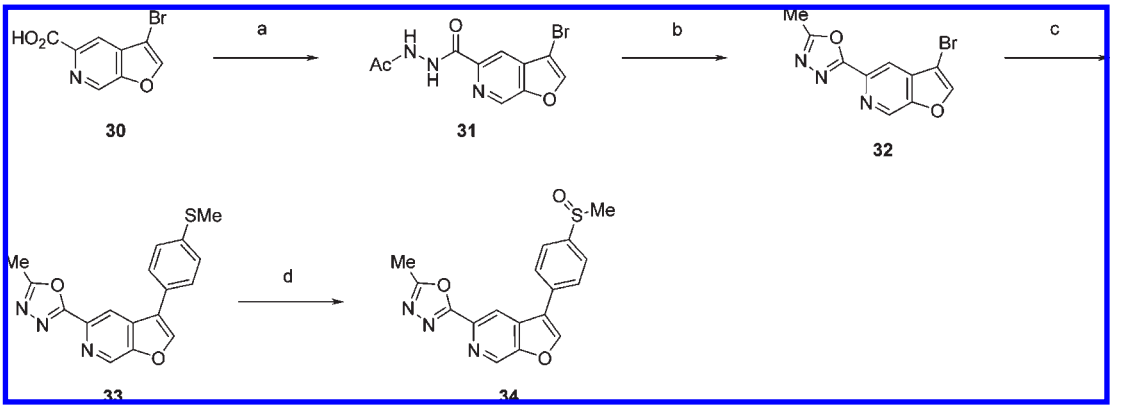


Het	24	25	26	27 - 29
a	R = Me	R = Me X = Br	R = Me	-
b	R = Me	R = Me X = I	R = Me	-
c	R = Et	R = Et X = Br	R = Et	-
d	R = Et	R = Et X = Br	R = Et	-

[a] Reagents and conditions: (a) NIS, CH_3CN, 80 °C, 73% (**25b**); (b) (1) Br_2, AcOH or CH_2Cl_2, (2) KOH, THF-EtOH, 0 °C, 85% (**25a**), 36% (**25c**), 30% (**25d**); (c) 4-(methylthio)phenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, DME, H_2O, reflux, 32−95%; (d) $NH_2NH_2 \cdot H_2O$, MeOH or EtOH, reflux, 49−95%; (e) $CH_3C(OEt)_3$, 120 °C, 93% (**28a**); (f) $CH_3C(OEt)_3$, DBU, *n*-BuOH, reflux, 67% (**28c**); (g) (1) AcCl, DMA, (2) TsCl, pyridine, 100 °C, 29% (**28b**), 58% (**28d**); (h) *m*-CPBA, CH_2Cl_2, 41−86%.

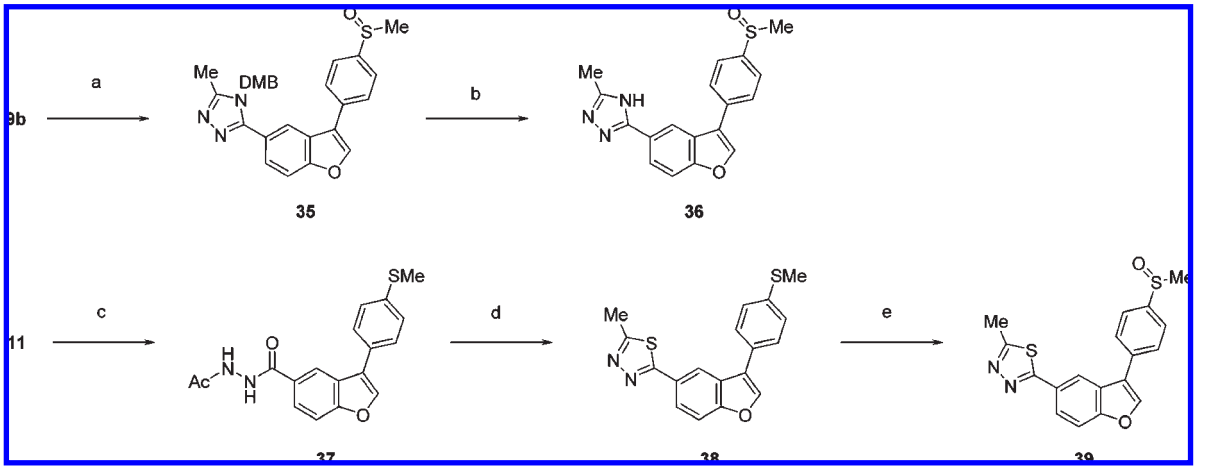
activity to **2**. However, the bioavailability and solubility of **8e** and **8f** were improved. Although phenol **8l**, ketone **8j**, alcohol **8k**, ester **8d**, dimethyl phosphonate **8m**, and 4-fluorophenyl derivative **8c** were less active than **2**, 4-methylthio derivative **8a**, methylsulfinyl derivative **9b**, and methylsulfonyl derivative **8n** exhibited equipotent or more potent activities with IC_{50} values of 66, 35, and 42 nM, respectively. These results point to the importance of electrostatic interaction with Arg141 in determining inhibitory activity. Among these compounds, methylsulfinyl derivative **9b** exhibited good solubility (38 μg/mL) and bioavailability ($F = 40\%$). Replacing the methyl group of **9b** or **8n** with an ethyl group resulted in similar inhibitory potency (**9c**,**d**), with **9c** showing good solubility (62 μg/mL) and bioavailability ($F =$

Scheme 5[a]



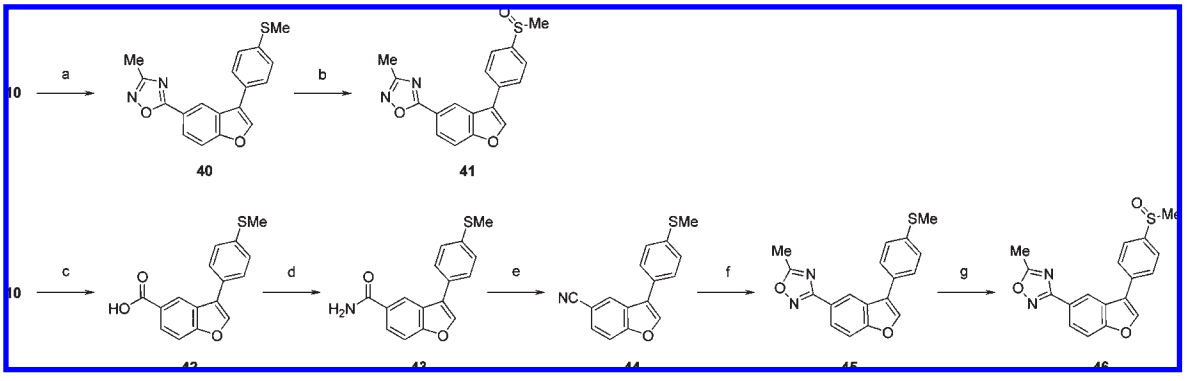
[a] Reagents and conditions: (a) $AcNHNH_2$, EDC, HOBt, DMF, 28%; (b) TsCl, pyridine, 90 °C, 42%; (c) 4-(methylthio)phenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, DME, H_2O, reflux, 52%; (d) *m*-CPBA, CH_2Cl_2, 67%.

Scheme 6[a]



[a] Reagents and conditions: (a) 2,4-dimethoxybenzylamine, *p*-TsOH, xylene, 150 °C, 44%; (b) TFA, rt, 39%; (c) AcOH, EDC, HOBt, DMF, 75%; (d) Lawesson's reagent, toluene, 80 °C, 54%; (e) *m*-CPBA, CH_2Cl_2, 93%.

Scheme 7[a]

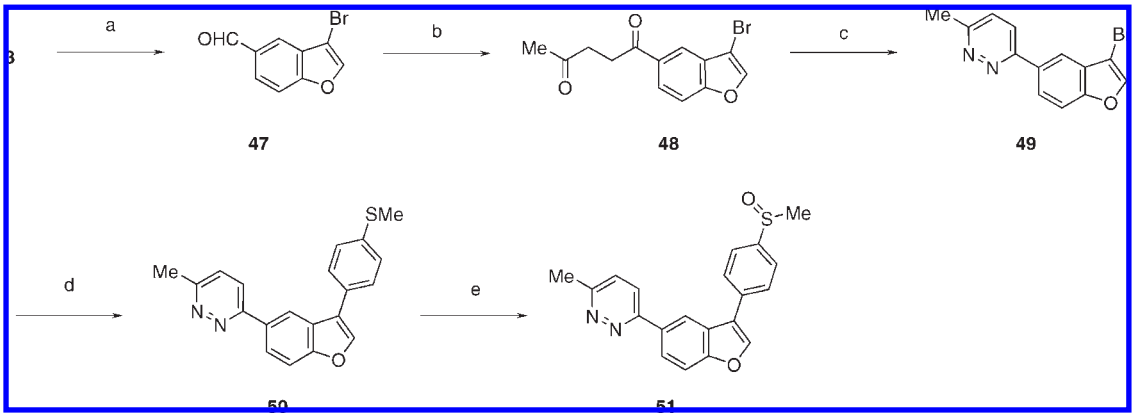


[a] Reagents and conditions: (a) acetamidoxime, NaH, THF, 60 °C, 67%; (b) *m*-CPBA, CH_2Cl_2, 91%; (c) 1M NaOH aq, MeOH-THF, 96%; (d) (1) EDC, HOBt, DMF; (2) aq NH_3, rt, 95%; (e) $SOCl_2$, DMF, 76%; (f) (1) $NH_2OH \cdot HCl$, $NaHCO_3$, MeOH, reflux; (2) Ac_2O, dioxane, 90 °C, 76%; (g) *m*-CPBA, CH_2Cl_2, 87%.

85%). Conversion of the phenyl ring to pyridine showed similar activity, improved solubility, and bioavailability compared to **2**. Shifting the methoxy or sulfinyl group at the 4-postion to the 3-position resulted in decreased activity (**8h**, **9a**). It was observed that phenyl-substituted compounds with lower log *D* values generally showed improved solubility and metabolic stability. Because 4-methylsulfinyl phenyl derivative **9b** showed good solubility and bioavailability, we modified other parts of **9b**.

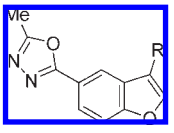
The effect of converting the benzofuran ring to other heterocycles is shown in Table 2. Despite our expectations, based on previous studies, that benzimidazole would be a more suitable hinge binder, compound **23** showed a 5-fold decrease in activity. It is possible that tighter binding of the

Scheme 8[a]



[a] Reagents and conditions: (a) (1) NBS, AIBN, PhCl, 80 °C, 83%, (2) Br_2, CH_2Cl_2 then KOH, EtOH, 0 °C, 44%; (b) methyl vinyl ketone, NEt_3, 3-ethyl-5-(2-hydroxyethyl)-4-methylthiazolium bromide EtOH, 70 °C, 25%; (c) $NH_2NH_2 \cdot H_2O$, EtOH, reflux, 23%; (d) [4-(methylthio)phenyl]boronic acid, Na_2CO_3, $Pd(PPh_3)_4$, DME, H_2O, reflux, 68%; (e) *m*-CPBA, CH_2Cl_2, 87%.

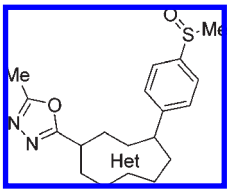
Table 1. Effect of Substitutions on Phenyl Ring

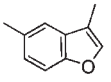
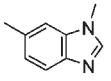
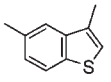
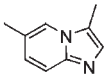
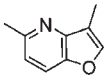
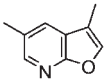
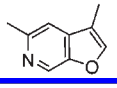


compd	R	IC_{50} (nM)[a]	log D[b]	metabolic stability human[c] (mL/min/mg)	Solubility[d] (μg/mL)	F[e] (%)
2	Ph-4-OMe	54	3.39	179	0.14	nd[f]
8e	Ph-4-CO_2H	50	1.18	0	54	28
8f	Ph-4-$CONH_2$	69	1.67	121	32	32
8l	Ph-4-OH	180	2.47	170	1.1	nd[f]
8j	Ph-4-COMe	100	2.74	157	0.13	9.8
8k	Ph-4-C(OH)Me	160	2.68	85	26	6.6
8d	Ph-4-CO_2Me	200	3.22	248	0.07	nd[f]
8m	Ph-4-P(O)$(OMe)_2$	280	2.28	0	31	44
8c	Ph-4-F	110	3.32	155	1.0	nd[f]
8a	Ph-4-SMe	66	3.86	75	0.06	1.4
9b	Ph-4-S(O)Me	35	1.75	7	38	40
8n	Ph-4-$S(O)_2$Me	42	1.69	48	0.49	28
9c	Ph-4-S(O)Et	35	2.13	10	62	85
9d	Ph-4-$S(O)_2$Et	38	2.04	53	2.4	52
8g	4-Py	77	1.87	67	33	21
8h	Ph-3-OMe	74	3.41	191	0.61	nd[f]
9a	Ph-3-S(O)Me	210	1.85	75	>61	10

[a] IC_{50} values for GSK-3β shown are the mean of duplicate or triplicate measurements. [b] Measured at pH 7.4. [c] Metabolic stability in human hepatic microsome [d] Measured at pH 6.8. [e] Bioavailability in a rat cassette dosing at 0.1 mg/kg iv and 1 mg/kg po. [f] Not detected.

Table 2. Effect of Hinge Binding Element



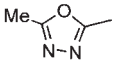
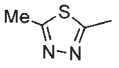
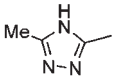
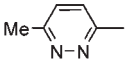
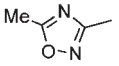
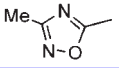
compd	Het	IC_{50} (nM)[a]
9b		35
23		200
29a		790
29b		>1000
29c		270
29d		>1000
34		>1000

[a] IC_{50} values for GSK-3β shown are the mean of duplicate or triplicate measurements.

benzimidazole ring with the hinge amide could negatively affect interactions between the oxadiazole nitrogen and the conserved water or between the sulfoxide and Arg141. Benzothiophene **29a** and imidazo[1,2-*a*]pyridine derivative **29b** showed decrease in activity. Reduced electron density of the heterocycles and altered direction of the two side chains might affect the binding affinities as well. In the furopyridine series, furo[3,2-*b*]pyridine **29c** showed moderate activity, while furo-[2,3-*b*]pyridine **29d** and furo[2,3-*c*]pyridine **34** exhibited markedly decreased activity.

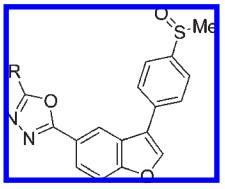
Substitution of the 1,3,4-oxadiazole ring and its impact on inhibitory activity is displayed in Table 3. Interestingly, 1,3,4-thiadiazole **39** and 1,2,4-triazole **36** showed slightly decreased activity compared to 1,3,4-oxadiazole **9b**. However, pyridazine **51** showed markedly reduced potency, as did isomers of **9b**, 1,2,4-oxadiazoles **46**, **41**. These results suggest that the adjacent nitrogen atom and ring size are closely fit to the binding pocket. Reduced potency of compounds **46** and **41** might be due to reduced electron density of the nitrogen atom, which resulted in weak hydrogen bond with the binding sites.

Table 3. Effect of Heterocyclic Rings

compd	Ar	IC_{50} (nM)[a]
9b		35
39		72
36		81
51		>1000
46		680
41		>1000

[a] IC_{50} values for GSK-3β shown are the mean of duplicate measurements.

Table 4. Effect of Substituents on the Oxadiazole



compd	R	IC_{50} (nM)[a]
9b	Me	35
15	SH	94
13a	OH	130
13b	NH_2	13

[a] IC_{50} values for GSK-3β shown are the mean of duplicate or triplicate measurements.

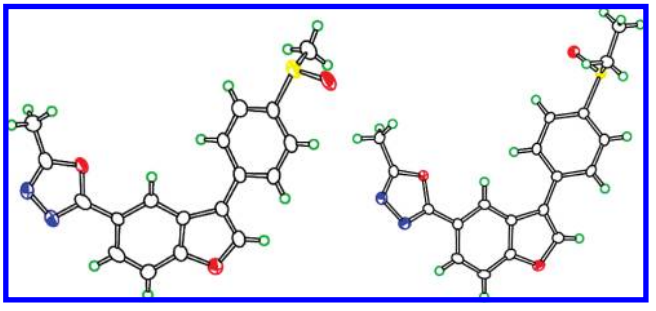
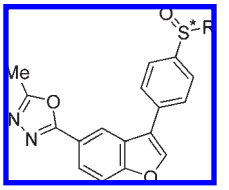


Figure 3. X-ray crystal structures of (*S*)-**9b** and (*S*)-**9c**.

Table 5. Inhibitory Activity of Enantiomers



compd	R	IC_{50} (nM)[a]
(*R*)-**9b**	Me	140
(*S*)-**9b**	Me	34
(*R*)-**9c**	Et	190
(*S*)-**9c**	Et	20

[a] IC_{50} values for GSK-3β shown are the mean of duplicate measurements.

The effect of varying the methyl group on the oxadiazole ring is shown in Table 4. Mercapto- and hydroxy-oxadiazoles **15**, **13a** showed reduced activity, while amino-oxadiazole **13b** showed 3-fold more potent activity compared to **9b**. A docking study suggested that the amino group interacts with the carboxyl group of Asp200. Unfortunately, amino-oxadiazole **13b** had poor oral bioavailability, probably due to low solubility and absorption (data not shown). We found that in our newly designed scaffold (**I**) the introduction of appropriate polar substituents at the 4-position of the phenyl ring improves inhibitory activity and biological properties, and the introduction of a five-membered azole with an adjacent nitrogen is suitable for interaction with the hydrogen bond network composed of the surrounding amino acids (e.g., Lys85, Glu97, Asp200) and conserved water molecules. On the basis of these results, sulfoxide derivatives **9b** and **9c** were selected for further evaluation.

We measured the potencies for the enantiomers of **9b** and **9c**. Optical resolution of the racemates **9b** and **9c** was carried out on a chiral column by high performance liquid chromatography (HPLC). Absolute configurations were confirmed by X-ray crystallographic analysis of (*S*)-**9b** and (*S*)-**9c** as illustrated in Figure 3.

The S-isomers (*S*)-**9b**, (*S*)-**9c** were found to be eutomers as shown in Table 5.

Pharmacokinetic profiles in rats and brain concentrations in mice of (*S*)-**9b** and (*S*)-**9c** were evaluated and the results are shown in Table 6. (*S*)-**9b** and (*S*)-**9c** possessed good oral absorption in rats with bioavailability (*F* %) of 72.8% and 65.5%, respectively. In addition, when (*S*)-**9b** and (*S*)-**9c** were given orally at a dose of 3 mg/kg in mice, AUC_{0-24h}s were 734 and 547 ng·h/g, and K_p values (ratio of brain and plasma) were 1.6 and 1.3, respectively (Table 6). Thus, these compounds exhibited good pharmacokinetic profiles and favorable BBB permeability.

To determine kinase selectivity profiles, (*S*)-**9b** and (*S*)-**9c** were tested for inhibitory activity against 10 serine/threonine kinases and 12 tyrosine kinases. Compounds (*S*)-**9b** and (*S*)-**9c** showed no significant activity and IC_{50} values were greater than 10 μM, indicating that these compounds are highly selective GSK-3β inhibitors (Table 7).

The X-ray cocrystal structure of (*S*)-**9b** was obtained, and analysis revealed that the N4 nitrogen atom of the oxadiazole ring formed a hydrogen bond with the side chain of Lys85 of the conserved Lys-Glu pair (Figure 4). The oxygen atom and

Table 6. Pharmacokinetic Parameters in Rats and Brain or Plasma Concentrations in Mice

	rats[a,b]						mice[a,c]	
compd	$V_{dss,iv}$ (mL/kg)	$CL_{total,iv}$ (mL/min/kg)	$C_{max,po}$ (ng/mL)	$AUC_{0-24h,po}$ (ng·h/mL)	MRT_{po} (h)	F^d (%)	brain (ng·h/g)	plasma (ng·h/mL)
(*S*)-**9b**	1134	27.4	396.9	1380.6	2.19	72.8	734	457
(*S*)-**9c**	1650	28.4	289.6	1229.1	3.03	65.5	547	409

[a] Data are expressed as the mean of three determinations. [b] Dosed at 1 mg/kg iv and 3 mg/kg po in Crj:CD (SD) IGS rats. [c] Dosed at 3 mg/kg po in C57BL/6N mice. [d] Bioavailability.

Table 7. Kinase Selectivity of (*S*)-**9b**, (*S*)-**9c**

	IC_{50} (nM)	
compd	Ser/Thr kinase CDK1, CDK2, CDK5, CHK1, p38α, JNK1, MEKK1, IKKβ, PKCθ, CK1δ	Tyr kinase EGFR, ERBB2, Src, Lck, IR, TIE2, c-Kit, c-Met, VEGFR2, FGFR3, PDGFRα, PDGFRβ
(*S*)-**9b**	> 10000	> 10000
(*S*)-**9c**	> 10000	> 10000

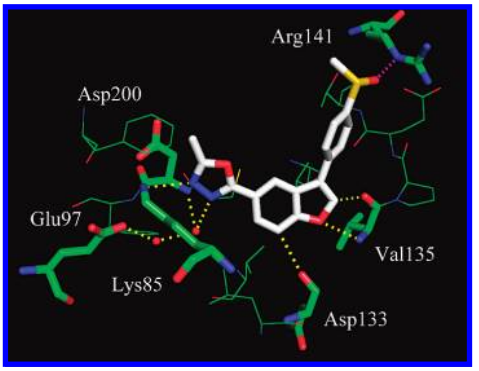


Figure 4. X-ray cocrystal structure of (*S*)-**9b** in complex with GSK-3β. The figure was prepared with PyMOL.[23]

the C2 hydrogen atom of the benzofuran ring interact with main chain of Val135 and the hydrogen atom at the C7 also interacts with carbonyl of Asp133 through a hydrogen bond. The sulfoxide on the phenyl ring of (*S*)-**9b** was revealed to interact with Arg141.

In vivo efficacy of (*S*)-**9b** and (*S*)-**9c** was examined using a cold water stress model (CWS) in mice.[30] We used the CWS model since recent work has demonstrated the involvement of GSK-3β in tau phosphorylation and the efficacy of lithium in this model.[30−32] CWS induced tau phosphorylation at several GSK-3β directed sites such as pS199, pThr205, pThr231, and pSer396. As the greatest increase in tau phosphorylation was detected by the pThr205 antibody in our initial experiments, we used this antibody for evaluating the efficacy of our compounds. Compounds (*S*)-**9b** and (*S*)-**9c** significantly reduced tau phosphorylation by 35% and 38%, respectively (Figure 5). We have observed that sulfoxide (*S*)-**9b** was partially oxidized to sulfone **8n** in plasma. However, oral administration (30 mg/kg) of sulfone **8n** did not significantly reduce tau phosphorylation in the CWS model.

Conclusion

In this report, we described the synthesis and evaluation of 3-aryl benzofuran derivatives based on the previously reported oxadiazole derivatives **1a** and **1b**. Removal of the S-benzyl group and optimization of the methoxy group interacting with

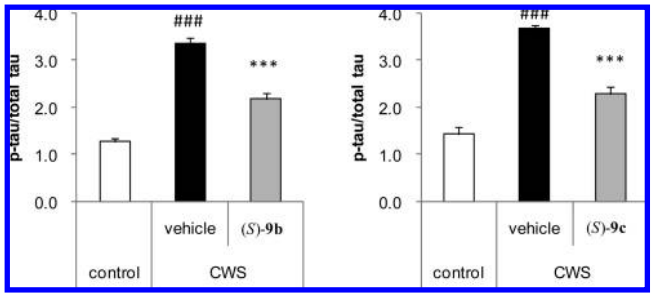


Figure 5. Effect of (*S*)-**9b** and (*S*)-**9c** on cold water stress (CWS) induced tau phosphorylation. (*S*)-**9b** and (*S*)-**9c** were orally administered at a dose of 3 mg/kg 30 min before the CWS. Immunoblot bands were quantified by densitometry. Data represent means ± SEM of 5 animals and are expressed as phospho-tau normalized to total tau. Significance is defined as ### $p \leq 0.001$ (Student's *t* test) in comparison to control group and as *** $p \leq 0.001$ (Student's *t* test) in comparison to CWS-treated control group.

Arg141 led to identification of sulfoxide derivatives **9b** and **9c**. Their eutomers (*S*)-**9b** and (*S*)-**9c** showed good pharmacokinetic profiles including favorable BBB penetration and good kinase selectivity. In addition, (*S*)-**9b** and (*S*)-**9c** administered orally to mice significantly inhibited hyperphosphorylation of tau protein in a CWS model. On the basis of these results, compounds (*S*)-**9b** and (*S*)-**9c** were selected for further pharmacological evaluation.

Experimental Section

Melting points were determined on a Buchi melting point apparatus and were not corrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Varian Gemini-300 (300 MHz) or Bruker DPX300 (300 MHz) instruments. Chemical shifts are reported as δ values (ppm) downfield from internal tetramethylsilane of the indicated organic solution. Peak multiplicities are expressed as follows. Abbreviations are used as follows: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublet; ddd, doublet of doublet of doublets; dt, doublet of triplet; brs, broad singlet; m, multiplet. Coupling constants (*J* values) are given in hertz (Hz). Element analyses were carried out by Takeda Analytical Laboratories, and the results were within 0.4% of theoretical values. LC/MS (ESI^+) was performed on a ZQ-2000 apparatus with acetonitrile/water mobile phase. Purities of test compounds (**15**, **29d**) were established by analytical HPLC using the following conditions: column, Imtakt Cadenza CD-C18, 2.0 mm × 50 mm, S-3 μm; flow rate of 0.5 mL/min; solvent, H_2O/0.1% TFA (A), MeCN/0.1% TFA (B); gradient condition: 10% B for 0.01 min, linear increase from 10% to 95% B over 4 min, 100% B for 1.5 min. Analytical HPLC was equipped with a LC-10ADvp pump and SPD-10A-vp (UV detector) set at 254 and 220 nm. Preparative HPLC were performed an automated Gilson HPLC system using a YMC C-18 column (S-5 μM 50 mm × 20 mm i.d.) with 10−100% gradient water−acetonitrile containing 0.1% TFA. Reaction progress was determined by thin layer chromatography (TLC)

analysis on silica gel 60 F_{254} plate (Merck) or NH TLC plates (Fuji Silysia Chemical Ltd.).

Methyl 2,3-dihydro-1-benzofuran-5-carboxylate (4). To a solution of **3** (33.9 g, 229 mmol) and KOH (85%, 39.3 g, 596 mmol) in MeOH (250 mL) at 0 °C was slowly added a solution of iodine (75.6 g, 298 mmol) in MeOH (750 mL), and the mixture was stirred overnight at room temperature. The reaction mixture was diluted with EtOAc, and the organic layer was washed with 1 M sodium sulfite, water, and brine, dried over magnesium sulfate, and concentrated in vacuo. The residue was purified by basic silica gel column chromatography (hexane/EtOAc = 5/1), and the product was recrystallized from hexane/EtOAc to give **4** (35.0 g, 86%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 3.24 (2H, t, J = 8.9 Hz), 3.87 (3H, s), 4.65 (2H, t, J = 8.9 Hz), 6.79 (1H, d, J = 8.3 Hz), 7.85−7.89 (2H, m).

Methyl 3-Bromo-1-benzofuran-5-carboxylate (5). A mixture of **4** (35.0 g, 196.4 mmol), NBS (38.9 g, 216.0 mmol), and AIBN (0.645 g, 3.93 mmol) in PhCl (350 mL) was stirred at 85 °C for 2 h. The reaction mixture was cooled to room temperature, diluted with EtOAc, and the organic layer was washed with water, saturated aqueous sodium hydrogen carbonate, and brine, dried over magnesium sulfate, and concentrated in vacuo. The residue was purified by basic silica gel column chromatography (hexane/EtOAc = 5/1) to give methyl 1-benzofuran-5-carboxylate. To a mixture of methyl 1-benzofuran-5-carboxylate in CH_2Cl_2 (150 mL) at 0 °C was slowly added bromine (10.1 mL, 196.4 mmol), and the mixture was stirred at room temperature for 1 h. The organic layer was washed with 1 M sodium sulfite and brine, dried over magnesium sulfate, and concentrated in vacuo to give methyl 2,3-dibromo-2,3-dihydro-1-benzofuran-5-carboxylate. To a solution of methyl 2,3-dibromo-2,3-dihydro-1-benzofuran-5-carboxylate in THF (250 mL) at 0 °C was added a mixture of KOH (85%, 13.0 g, 196.4 mmol) in MeOH (50 mL), and the mixture was stirred for 15 min. The reaction mixture was diluted with EtOAc, and the organic layer was washed with water and saturated aqueous sodium hydrogen carbonate, dried over magnesium sulfate, and concentrated in vacuo. The residue was purified by basic silica gel column chromatography (THF), and the product was recrystallized from hexane/EtOAc to give **5** (44.6 g, 89%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 3.97 (3H, s), 7.53 (1H, dd, J = 0.8, 8.7 Hz), 7.72 (1H, s), 8.09 (1H, dd, J = 1.9, 8.7 Hz), 8.30 (1H, dd, J = 0.8, 1.9 Hz).

3-Bromo-1-benzofuran-5-carbohydrazide (6). A solution of **5** (30.6 g, 120 mmol) and hydrazine hydrate (29.1 mL, 0.59 mol) in MeOH (300 mL) was refluxed for 24 h. The reaction mixture was cooled to 0 °C, and precipitated solid was filtered and washed with cold MeOH to give **6** (29.2 g, 95%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 4.53 (2H, brs), 7.74 (1H, dd, J = 0.6, 8.7 Hz), 7.92 (1H, dd, J = 1.7, 8.7 Hz), 8.07 (1H, dd, J = 0.6, 1.7 Hz), 8.39 (1H, s), 9.92 (1H, brs).

2-(3-Bromo-1-benzofuran-5-yl)-5-methyl-1,3,4-oxadiazole (7). A solution of **6** (25.5 g, 100 mmol) in triethyl orthoacetate (150 mL) was stirred at 120 °C for 24 h. The reaction mixture was cooled to room temperature, and precipitated solid was filtered and washed with EtOAc. The solid was purified by basic silica gel (THF), and the product was washed with hexane/THF (1/1) to give **7** (27.0 g, 97%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 2.65 (3H, s), 7.62 (1H, dd, J = 0.6, 8.9 Hz), 7.75 (1H, s), 8.09 (1H, dd, J = 1.7, 8.9 Hz), 8.24 (1H, dd, J = 0.6, 1.7 Hz).

2-[3-(4-Methoxyphenyl)-1-benzofuran-5-yl]-5-methyl-1,3,4-oxadiazole (2). A solution of **7** (0.25 g, 0.90 mmol), 4-methoxyphenylboronic acid (0.15 g, 1.00 mmol), sodium carbonate (0.21 g, 2.00 mmol), and tetrakis(triphenylphosphine)palladium (31.2 mg, 0.027 mmol) in DME (5 mL) and water (1 mL) under nitrogen atmosphere was refluxed overnight. To the reaction mixture was added water and extracted with EtOAc. The combined organic layer was washed with brine, dried over sodium sulfate, and concentrated in vacuo. The residue was purified by silica gel column chromatography (EtOAc), and the product was recrystallized from hexane/EtOAc to give **2** (0.21 g, 76%) as a colorless solid.

^{1}H NMR ($CDCl_3$) δ 2.64 (3H, s), 3.88 (3H, s), 7.02−7.08 (2H, m), 7.56−7.61 (2H, m), 7.64 (1H, d, J = 8.7 Hz), 7.80 (1H, s), 8.04 (1H, dd, J = 1.5, 8.7 Hz), 8.48 (1H, d, J = 1.5 Hz). Anal. ($C_{18}H_{14}N_2O_3$) C, H, N.

The following compounds **8a**−**d**,**f**−**j**,**l**,**n** were prepared in a manner similar to that described for **2**.

2-Methyl-5-{3-[4-(methylsulfanyl)phenyl]-1-benzofuran-5-yl}-1,3,4-oxadiazole (8a). Yield 71%, colorless solid. ^{1}H NMR ($CDCl_3$) δ 2.55 (3H, s), 2.64 (3H, s), 7.37−7.42 (2H, m), 7.57−7.61 (2H, m), 7.65 (1H, dd, J = 0.8, 8.7 Hz), 7.85 (1H, s), 8.06 (1H, dd, J = 1.7, 8.7 Hz), 8.48 (1H, dd, J = 0.6, 1.7 Hz). Anal. ($C_{18}H_{14}N_2O_2S$) C, H, N.

2-{3-[4-(Ethylsulfanyl)phenyl]-1-benzofuran-5-yl}-5-methyl-1,3,4-oxadiazole (8b). Yield 72%, colorless solid. ^{1}H NMR ($CDCl_3$) δ 1.38 (3H, t, J = 7.3 Hz), 2.64 (3H, s), 3.02 (2H, q, J = 7.3 Hz), 7.46 (2H, d, J = 8.5 Hz), 7.58 (2H, d, J = 8.5 Hz), 7.65 (1H, dd, J = 0.8, 8.7 Hz), 7.86 (1H, s), 8.05 (1H, dd, J = 1.7, 8.7 Hz), 8.49 (1H, d, J = 1.7 Hz). Anal. ($C_{19}H_{16}N_2O_2S$) C, H, N.

2-[3-(4-Fluorophenyl)-1-benzofuran-5-yl]-5-methyl-1,3,4-oxadiazole (8c). Yield 69%, colorless solid. ^{1}H NMR ($CDCl_3$) δ 2.64 (3H, s), 7.17−7.35 (2H, m), 7.59−7.67 (3H, m), 7.83 (1H, s), 8.06 (1H, dd, J = 1.7, 8.7 Hz), 8.45 (1H, dd, J = 0.6, 1.7 Hz). Anal. ($C_{17}H_{11}FN_2O_2$) C, H, N.

Methyl 4-[5-(5-Methyl-1,3,4-oxadiazol-2-yl)-1-benzofuran-3-yl]benzoate (8d). Yield 64%, colorless solid. ^{1}H NMR ($CDCl_3$) δ 2.65 (3H, s), 3.97 (3H, s), 7.68 (1H, dd, J = 0.6, 8.7 Hz), 7.73−7.77 (2H, m), 7.95 (1H, s), 8.09 (1H, dd, J = 1.7, 8.7 Hz), 8.16−8.20 (2H, m), 8.51 (1H, dd, J = 0.6, 1.7 Hz). Anal. ($C_{19}H_{14}N_2O_4$) C, H, N.

4-[5-(5-Methyl-1,3,4-oxadiazol-2-yl)-1-benzofuran-3-yl]benzoic acid (8e). To a solution of **8d** (204 mg, 0.610 mmol) in THF (6 mL) and MeOH (6 mL) was added 1 M NaOH (3 mL), and the mixture was stirred at 60 °C for 24 h. After the reaction mixture was cooled to room temperature, acidified with 1 M HCl, and precipitated solid was collected. The crude solid was recrystallized from EtOH to give **8e** (163 mg, 83%) as a colorless solid.

^{1}H NMR (DMSO-d_6) δ 2.61 (3H, s), 7.89−7.94 (3H, m), 8.05 (1H, dd, J = 1.7, 8.7 Hz), 8.10−8.14 (2H, m), 8.46 (1H, dd, J = 0.6, 1.7 Hz), 8.67 (1H, s), 13.08 (1H, brs). Anal. ($C_{18}H_{12}N_2O_4$) C, H, N.

4-[5-(5-Methyl-1,3,4-oxadiazol-2-yl)-1-benzofuran-3-yl]benzamide (8f). Yield 65%, colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.61 (3H, s), 7.46 (1H, brs), 7.84−7.88 (2H, m), 7.92 (1H, dd, J = 0.6, 8.7 Hz), 8.03−8.08 (4H, m), 8.44 (1H, dd, J = 0.6, 1.7 Hz), 8.63 (1H, s). Anal. ($C_{18}H_{13}N_3O_3$) C, H, N.

4-[5-(5-Methyl-1,3,4-oxadiazol-2-yl)-1-benzofuran-3-yl]pyridine (8g). Yield 42%, colorless solid. ^{1}H NMR ($CDCl_3$) δ 2.66 (3H, s), 7.60 (2H, dd, J = 1.5, 4.5 Hz), 7.70 (1H, dd, J = 0.6, 8.7 Hz), 8.03 (1H, s), 8.11 (1H, dd, J = 1.7, 8.7 Hz), 8.54 (1H, dd, J = 0.6, 1.7 Hz), 8.75 (2H, d, J = 5.1 Hz). Anal. ($C_{16}H_{11}N_3O_2$) C, H, N.

2-[3-(3-Methoxyphenyl)-1-benzofuran-5-yl]-5-methyl-1,3,4-oxadiazole (8h). Yield 77%, colorless solid. ^{1}H NMR ($CDCl_3$) δ 2.64 (3H, s), 3.90 (3H, s), 6.97 (1H, ddd, J = 0.9, 2.6, 8.3 Hz), 7.18 (1H, dd, J = 1.5, 2.6 Hz), 7.26 (1H, ddd, J = 0.9, 1.5, 7.5 Hz), 7.44 (1H, dd, J = 7.5, 8.3 Hz), 7.65 (1H, dd, J = 0.6, 8.7 Hz), 7.86 (1H, s), 8.07 (1H, dd, J = 1.7, 8.7 Hz), 8.50 (1H, dd, J = 0.6, 1.7 Hz). Anal. ($C_{18}H_{14}N_2O_3$) C, H, N.

2-Methyl-5-{3-[3-(methylsulfanyl)phenyl]-1-benzofuran-5-yl}-1,3,4-oxadiazole (8i). Yield 88%, colorless solid. ^{1}H NMR ($CDCl_3$) δ 2.56 (3H, s), 2.64 (3H, s), 7.27−7.34 (1H, m), 7.42−7.46 (2H, m), 7.50−7.51 (1H, m), 7.66 (1H, dd, J = 0.8, 8.7 Hz), 7.86 (1H, s), 8.07 (1H, dd, J = 1.7, 8.7 Hz), 8.46 (1H, dd, J = 0.6, 1.7 Hz). Anal. ($C_{18}H_{14}N_2O_2S$) C, H, N.

1-{4-[5-(5-Methyl-1,3,4-oxadiazol-2-yl)-1-benzofuran-3-yl]-phenyl}ethanone (8j). Yield 79%, colorless solid. ^{1}H NMR ($CDCl_3$) δ 2.65 (3H, s), 2.67 (3H, s), 7.69 (1H, dd, J = 0.6, 8.7 Hz), 7.75−7.80 (2H, m), 7.96 (1H, s), 8.06−8.14 (3H, m), 8.50−8.56 (1H, m). Anal. ($C_{19}H_{14}N_2O_3$) C, H, N.

1-{4-[5-(5-Methyl-1,3,4-oxadiazol-2-yl)-1-benzofuran-3-yl]-phenyl}ethanol (8k). To a solution of **8j** (0.30 g, 0.94 mmol) in EtOH (3 mL) at 0 °C was added sodium tetrahydroborate (90%, 59 mg, 1.41 mmol) and the mixture was stirred overnight at room temperature. The mixture was concentrated, diluted with water, and extracted with EtOAc. The organic layer was washed with brine, dried over magnesium sulfate, and concentrated in vacuo. The residue was purified by basic silica gel column chromatography (hexane/EtOAc = 1/2 to 1/3), and the product was recrystallized from hexane/EtOAc to give **8k** (0.23 g, 76%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 1.58 (3H, d, J = 0.8 Hz), 1.86−1.94 (1H, m), 2.57−2.68 (3H, m), 4.94−5.05 (1H, m), 7.53 (2H, d, J = 7.9 Hz), 7.61−7.69 (3H, m), 7.86 (1H, s), 8.05 (1H, dd, J = 1.5, 8.7 Hz), 8.49 (1H, d, J = 1.5 Hz). Anal. ($C_{19}H_{16}N_2O_3$) C, H, N.

4-[5-(5-Methyl-1,3,4-oxadiazol-2-yl)-1-benzofuran-3-yl]phenol (8l). Yield 95%, colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.60 (3H, s), 6.95 (2H, d, J = 8.3 Hz), 7.56 (2H, d, J = 8.3 Hz), 7.86 (1H, d, J = 8.7 Hz), 8.00 (1H, dd, J = 1.7, 8.7 Hz), 8.36 (2H, s). Anal. ($C_{17}H_{12}N_2O_3 \cdot 0.2H_2O$) C, H, N.

Dimethyl {4-[5-(5-Methyl-1,3,4-oxadiazol-2-yl)-1-benzofuran-3-yl]phenyl}phosphonate (8m). To a solution of **8l** (380 mg, 1.30 mmol) and Tf_2NPh (511 mg, 1.43 mmol) in THF (15 mL) was slowly added a 60% suspension of sodium hydride in mineral oil (57.2 mg, 1.43 mmol), and the mixture was stirred for 30 min at room temperature. The reaction mixture was poured into water and extracted with EtOAc. The organic layer was washed with brine, dried over magnesium sulfate, and concentrated in vacuo. The residue was purified by basic silica gel column chromatography (hexane/THF = 1/2), and the product was recrystallized from hexane/THF to give 4-[5-(5-methyl-1,3,4-oxadiazol-2-yl)-1-benzofuran-3-yl]phenyl trifluoromethanesulfonate (507 mg, 92%) as a colorless solid.

A mixture of 4-[5-(5-methyl-1,3,4-oxadiazol-2-yl)-1-benzofuran-3-yl]phenyl trifluoromethanesulfonate (391 mg, 0.90 mmol), dimethyl phosphate (0.165 mL, 1.80 mmol), tetrakis(triphenylphosphine)palladium (104 mg, 0.09 mmol), and *N*-ethyldiisopropylamine (0.314 mL, 1.80 mmol) in toluene (5 mL) under argon atmosphere was stirred for 2 h at 100 °C. After cooling to room temperature, the mixture was diluted with water and extracted with EtOAc. The organic layer was washed with 1 M HCl and brine, dried over magnesium sulfate, and concentrated in vacuo. The residue was purified by basic silica gel column chromatography (EtOAc), and the product was recrystallized from hexane/EtOAc to give **8m** (190 mg, 55%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 2.65 (3H, s), 3.82 (6H, d, J = 11.1 Hz), 7.68 (1H, dd, J = 0.6, 8.7 Hz), 7.76−7.81 (2H, m), 7.91−7.99 (3H, m), 8.09 (1H, dd, J = 1.7, 8.7 Hz), 8.50 (1H, dd, J = 0.6, 1.7 Hz). Anal. ($C_{19}H_{17}N_2O_5P$) C, H, N.

2-Methyl-5-{3-[4-(methylsulfonyl)phenyl]-1-benzofuran-5-yl}-1,3,4-oxadiazole (8n). Yield 85%, colorless solid. ^{1}H NMR ($CDCl_3$) δ 2.65 (3H, s), 3.13 (3H, s), 7.70 (1H, dd, J = 0.6, 8.7 Hz), 7.85−7.89 (2H, m), 7.98 (1H, s), 8.08−8.12 (3H, m), 8.50 (1H, dd, J = 0.6, 1.7 Hz). Anal. ($C_{18}H_{14}N_2O_4S$) C, H, N.

2-Methyl-5-{3-[3-(methylsulfinyl)phenyl]-1-benzofuran-5-yl}-1,3,4-oxadiazole (9a). To a solution of **8i** (774 mg, 2.40 mmol) in CH_2Cl_2 (15 mL) was added 69−75% *m*-CPBA (888 mg, 3.60 mmol), and the mixture was stirred for 5 min at room temperature. The reaction mixture was concentrated in vacuo, and the residue was purified by basic silica gel column chromatography (EtOAc) and the product was recrystallized from CH_2Cl_2/MeOH to give **9a** (503 mg, 59%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 2.65 (3H, s), 3.14 (3H, s), 7.70 (1H, dd, J = 0.6, 8.7 Hz), 7.73−7.78 (1H, m), 7.97−8.01 (3H, m), 8.10 (1H, dd, J = 1.7, 8.7 Hz), 8.18−8.19 (1H, m), 8.46 (1H, dd, J = 0.6, 1.7 Hz). Anal. ($C_{18}H_{14}N_2O_3S$) C, H, N.

2-Methyl-5-{3-[4-(methylsulfinyl)phenyl]-1-benzofuran-5-yl}-1,3,4-oxadiazole (9b). Compound **9b** was prepared in a manner similar to that described for **9a** in 84% yield as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 2.65 (3H, s), 2.81 (3H, s), 7.69 (1H, dd, J = 0.6, 8.7 Hz), 7.78−7.85 (4H, m), 7.94 (1H, s), 8.08 (1H, dd, J = 1.7, 8.7 Hz), 8.50 (1H, dd, J = 0.6, 1.7 Hz). Anal. ($C_{18}H_{14}N_2O_3S$) C, H, N.

2-{3-[4-(Ethylsulfinyl)phenyl]-1-benzofuran-5-yl}-5-methyl-1,3,4-oxadiazole (9c). Compound **9c** was prepared in a manner similar to that described for **9a** in 82% yield as a colorless solid. ^{1}H NMR ($CDCl_3$) δ1.27 (3H, t, J = 7.3 Hz), 2.65 (3H, s), 2.78−3.06 (2H, m), 7.68 (1H, d, J = 8.7 Hz), 7.73−7.78 (2H, m), 7.79−7.85 (2H, m), 7.94 (1H, s), 8.08 (1H, dd, J = 1.7, 8.7 Hz), 8.51 (1H, d, J = 1.1 Hz). Anal. ($C_{19}H_{16}N_2O_3S$) C, H, N.

2-{3-[4-(Ethylsulfonyl)phenyl]-1-benzofuran-5-yl}-5-methyl-1,3,4-oxadiazole (9d). To a solution of **8b** (0.30 g, 0.89 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added 69−75% *m*-CPBA (0.63 g, 2.67 mmol), and the mixture was stirred for 4 days at room temperature. The reaction mixture was concentrated and purified by basic silica gel column chromatography (hexane/EtOAc = 1/1), and the product was recrystallized from hexane/EtOAc to give **9d** (86 mg, 26%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 1.35 (3H, t, J = 7.4 Hz), 2.65 (3H, s), 3.19 (2H, q, J = 7.3 Hz), 7.70 (1H, dd, J = 0.6, 8.7 Hz), 7.87 (2H, d, J = 8.5 Hz), 7.98 (1H, s), 8.05 (2H, d, J = 8.5 Hz), 8.10 (1H, dd, J = 1.7, 8.7 Hz), 8.49−8.51 (1H, m). Anal. ($C_{19}H_{16}N_2O_4S$) C, H, N.

Methyl 3-[4-(Methylsulfanyl)phenyl]-1-benzofuran-5-carboxylate (10). Compound **10** was prepared in a manner similar to that described for **2** in 85% yield as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 2.55 (3H, s), 3.95 (3H, s), 7.37−7.41 (2H, m), 7.55−7.60 (3H, m), 7.83 (1H, s), 8.08 (1H, dd, J = 1.7, 8.7 Hz), 8.53 (1H, dd, J = 0.6, 1.7 Hz). Anal. ($C_{17}H_{14}O_3S$) C, H, N.

3-[4-(Methylsulfanyl)phenyl]-1-benzofuran-5-carbohydrazide (11). A solution of **10** (7.46 g, 25.0 mmol) and hydrazine hydrate (3.06 mL, 125 mmol) in MeOH (100 mL) was refluxed for 3 days. After cooling, the precipitate was collected by filtration and recrystallized from MeOH to give **11** (7.11 g, 95%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.54 (3H, s), 4.52 (2H, brs), 7.40−7.44 (2H, m), 7.71−7.78 (3H, m), 7.89 (1H, dd, J = 1.7, 8.7 Hz), 8.38 (1H, dd, J = 0.4, 1.7 Hz), 8.45 (1H, s), 9.91 (1H, brs). Anal. ($C_{16}H_{14}N_2O_2S$) C, H, N.

5-{3-[4-(Methylsulfanyl)phenyl]-1-benzofuran-5-yl}-1,3,4-oxadiazol-2-ol (12a). To a solution of **11** (0.25 g, 0.83 mmol) and NEt_3 (0.17 mL, 1.25 mmol) in DMF (5 mL) at 0 °C was added CDI (0.27 g, 1.68 mmol), and the mixture was stirred at 0 °C for 1 h. The reaction mixture was diluted with water and extracted with EtOAc/THF (1/1) solution. The organic layer was washed with brine, dried over magnesium sulfate, and concentrated in vacuo. The residue was recrystallized from hexane/EtOAc to give **12a** (0.20 g, 74%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.54 (3H, s), 7.44 (2H, d, J = 8.6 Hz), 7.70 (2H, d, J = 8.6 Hz), 7.85 (2H, d, J = 1.1 Hz), 8.20 (1H, t, J = 1.1 Hz), 8.50 (1H, s), 12.59 (1H, brs). Anal. ($C_{17}H_{12}N_2O_3S$) C, H, N.

5-{3-[4-(Methylsulfanyl)phenyl]-1-benzofuran-5-yl}-1,3,4-oxadiazol-2-amine (12b). To a solution of **11** (1.60 g, 5.36 mmol) in DMA (25 mL) was added cyanogen bromide (0.62 g, 5.90 mmol), and the mixture was stirred for 6 h at room temperature. Then to the mixture was added cyanogen bromide (0.17 g, 1.61 mmol) and the mixture was stirred for 2 h at room temperature. The reaction mixture was diluted with water and precipitated solid was filtered. The solid was dissolved in EtOAc/THF (1/1) solution, then washed with brine, dried over magnesium sulfate, and concentrated in vacuo. The residue was purified by basic silica gel column chromatography (THF), and the product was recrystallized from hexane/THF to give **12b** (1.37 g, 79%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.54 (3H, s), 7.23 (2H, brs), 7.44 (2H, d, J = 8.3 Hz), 7.69 (2H, d, J = 8.3 Hz), 7.80−7.90 (2H, m), 8.21 (1H, brs), 8.47 (1H, s). Anal. ($C_{17}H_{13}N_3O_2S$) C, H, N.

5-{3-[4-(Methylsulfinyl)phenyl]-1-benzofuran-5-yl}-1,3,4-oxadiazol-2-ol (13a). Compound **13a** was prepared in a manner similar to that described for **9a** in 80% yield as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 2.81 (3H, s), 7.80−7.95 (4H, m), 7.96 (2H, d, J = 8.4 Hz), 8.24 (1H, s), 8.62 (1H, s), 12.59 (1H, s). Anal. ($C_{17}H_{12}N_2O_4S$) C, H, N.

5-{3-[4-(Methylsulfinyl)phenyl]-1-benzofuran-5-yl}-1,3,4-oxadiazol-2-amine (13b). To a solution of **12b** (0.25 g, 0.77 mmol) in THF (10 mL) at 0 °C was added oxone (0.24 g, 0.77 mmol) in water (3 mL), and the mixture was stirred at 0 °C for 30 min and at room temperature for 1 h. The reaction mixture was diluted with water, and precipitated solid was filtered and purified by reverse phase preparative HPLC to give **13b** (15 mg, 6%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.81 (3 H, s), 7.24 (2H, s), 7.84−7.92 (4H, m), 7.93−7.98 (2H, m), 8.25 (1H, s), 8.59 (1H, s). Anal. ($C_{17}H_{13}N_3O_3S$) C, H, N.

Methyl 3-[4-(Methylsulfinyl)phenyl]-1-benzofuran-5-carboxylate (14). The compound **14** was prepared in a manner similar to that described for **9a** in 93% yield as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 2.80 (3H, s), 3.96 (3H, s), 7.61 (1H, dd, J = 0.6, 8.9 Hz), 7.77−7.84 (4H, m), 7.92 (1H, s), 8.12 (1H, dd, J = 1.7, 8.9 Hz), 8.54 (1H, dd, J = 0.6, 1.7 Hz). Anal. ($C_{17}H_{14}O_4S$) C, H, N.

5-{3-[4-(Methylsulfinyl)phenyl]-1-benzofuran-5-yl}-1,3,4-oxadiazole-2-thiol (15). A solution of **14** (2.23 g, 7.10 mmol) and hydrazine hydrate (1.76 mmol, 36.3 mmol) in MeOH (25 mL) was refluxed for 4 days. The solvent was removed, and the residue was dissolved in EtOH (30 mL). To the solution were added carbon disulfide (1.28 mL, 21.3 mmol) and NEt_3 (1.19 mL, 8.52 mmol), and the mixture was refluxed for 4 h. The reaction mixture was cooled to room temperature, diluted with water and 1 M HCl, and extracted with EtOAc. The organic layer was washed with brine, dried over sodium sulfate, and concentrated in vacuo. The crude solid was recrystallized from THF to give **15** (1.19 g, 47%) as a pale-yellow solid. ^{1}H NMR (DMSO-d_6) δ 2.82 (3H, s), 7.85−7.89 (2H, m), 7.92 (1H, dd, J = 0.8, 8.9 Hz), 7.94−8.00 (3H, m), 8.33 (1H, dd, J = 0.8, 1.5 Hz), 8.65 (1H, s), 14.75 (1H, brs). HPLC purity: 96%

Methyl 3-{[4-(Methylsulfanyl)phenyl]amino}-4-nitrobenzoate (17). To a solution of **16** (10.64 g, 53.4 mmol) in DMSO (100 mL) was added 4-(methylthio)aniline (14.87 g, 106.8 mmol), and the mixture was stirred at 110 °C for 6 h. The reaction mixture was cooled to room temperature and diluted with EtOAc. The organic layer was washed with water and brine, dried over magnesium sulfate, and concentrated in vacuo. The crude solid was recrystallized from hexane/EtOAc to give **17** (15.88 g, 93%) as a brown solid. ^{1}H NMR ($CDCl_3$) δ 2.53 (3H, s), 3.89 (3H, s), 7.21 (2H, d, J = 8.7 Hz), 7.31−7.34 (3H, m), 7.85 (1H, d, J = 1.5 Hz), 8.25 (1H, d, J = 8.7 Hz), 9.42 (1H, s).

Methyl 4-Amino-3-{[4-(methylsulfanyl)phenyl]amino}benzoate (18). To a solution of sodium hydrosulfite (55.7 g, 320 mmol) in water (125 mL) at 0 °C was added dropwise a solution of **17** (6.37 g, 20.0 mmol) in THF (100 mL) and EtOH (50 mL), and the mixture was stirred at room temperature for 1 h. The reaction mixture was basified by saturated aqueous sodium hydrogen carbonate, concentrated, and the water layer was extracted with EtOAc. The organic layer was washed with saturated aqueous sodium hydrogen carbonate and brine, dried over sodium sulfate, and concentrated in vacuo. The residue was purified by basic silica gel column chromatography (hexane/EtOAc = 5/1 to 0/1) to give **18** (5.77 g, 100%) as a brown oil. ^{1}H NMR ($CDCl_3$) δ 2.43 (3H, s), 3.83 (3H, s), 4.22 (2H, s), 5.15 (1H, s), 6.66 (2H, d, J = 8.7 Hz), 6.76 (1H, d, J = 8.4 Hz), 7.22 (2H, d, J = 8.7 Hz), 7.73−7.79 (2H, m).

Methyl 1-[4-(Methylsulfanyl)phenyl]-1*H*-benzimidazole-6-carboxylate (19). A solution of **18** (5.78 g, 20.0 mmol) in formic acid (50 mL) was stirred overnight at 100 °C. After the mixture was cooled to room temperature, EtOAc was added and the organic layer was washed with saturated aqueous sodium hydrogen carbonate and brine, dried over magnesium sulfate, and concentrated in vacuo. The crude solid was recrystallized from hexane/EtOAc to give **19** (4.44 g, 74%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 2.58 (3H, s), 3.94 (3H, s), 7.42−7.48 (4H, m), 7.88 (1H, d, J = 8.4 Hz), 8.05 (1H, dd, J = 1.2, 7.2 Hz), 8.20 (2H, d, J = 5.2 Hz).

1-[4-(Methylsulfanyl)phenyl]-1*H*-benzimidazole-6-carbohydrazide (20). A solution of **19** (5.96 g, 20 mmol) and hydrazine hydrate (9.70 mL, 200 mmol) in EtOH (70 mL) was refluxed overnight. The reaction mixture was cooled to room temperature and precipitated solid was filtered and washed with EtOH to give **20** (5.14 g, 86%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.58 (3H, s), 4.47 (2H, s), 7.53 (2H, d, J = 8.7 Hz), 7.67 (2H, d, J = 8.7 Hz), 7.80 (2H, s), 8.06 (1H, s), 8.65 (1H, s), 9.83 (1H, s).

***N'*-Acetyl-1-[4-(methylsulfanyl)phenyl]-1*H*-benzimidazole-6-carbohydrazide (21).** To a solution of **20** (1.0 g, 3.35 mmol) in DMA (5 mL) at 0 °C was added acetyl chloride (0.26 mL, 3.69 mmol), and the mixture was stirred at room temperature for 1 h. To the mixture was added EtOAc and precipitated solid was collected and washed with EtOAc to give **21** (1.14 g, 100%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 1.92 (3H, s), 2.58 (3H, s), 7.53 (2H, d, J = 8.7 Hz), 7.69 (2H, d, J = 8.7 Hz), 7.87 (2H, s), 8.12 (1H, s), 8.82 (1H, s), 9.89 (1H, s), 10.38 (1H, s).

6-(5-Methyl-1,3,4-oxadiazol-2-yl)-1-[4-(methylsulfanyl)phenyl]-1*H*-benzimidazole (22). To a solution of **21** (761 mg, 2.24 mmol) in pyridine (10 mL) was added TsCl (1.56 g, 8.00 mmol), and the mixture was stirred overnight at 90 °C. The reaction mixture was cooled to room temperature, and the mixture was concentrated and diluted with chloroform. The organic layer was washed with saturated aqueous sodium hydrogen carbonate and brine, dried over sodium sulfate, and concentrated in vacuo. The residue was purified by basic silica gel (hexane/EtOAc = 4/1 to 1/1), and the product was recrystallized from hexane/EtOAc to give **22** (428 mg, 66%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 2.58 (3H, s), 2.63 (3H, s), 7.43−7.49 (4H, m), 7.95−8.02 (2H, m), 8.19 (2H, s). Anal. ($C_{17}H_{14}N_4OS\cdot 0.3H_2O$) C, H, N.

6-(5-Methyl-1,3,4-oxadiazol-2-yl)-1-[4-(methylsulfinyl)phenyl]-1*H*-benzimidazole (23). The compound **23** was prepared in a manner similar to that described for **9a** in 85% as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 2.64 (3H, s), 2.85 (3H, s), 7.74 (2H, d, J = 8.7 Hz), 7.94 (2H, d, J = 8.7 Hz), 7.98−8.05 (2H, m), 8.26−8.28 (2H, m). Anal. ($C_{17}H_{14}N_4O_2S\cdot 0.5H_2O$) C, H, N.

Methyl 3-Bromo-1-benzothiophene-5-carboxylate (25a). Bromine (2.06 mL, 40.2 mmol) was added dropwise to a stirred solution of **24a** (5.15 g, 26.8 mmol) in AcOH (50 mL), and the mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with EtOAc, and the organic layer was washed with 1 M sodium sulfite and saturated aqueous sodium hydrogen carbonate, dried over magnesium sulfate, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/EtOAc = 5/1), and the product was recrystallized from hexane/EtOAc to give **25a** (6.20 g, 85%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 3.99 (3H, s), 7.52 (1H, s), 7.90 (1H, dd, J = 0.6, 8.5 Hz), 8.08 (1H, ddd, J = 0.4, 1.5, 8.5 Hz), 8.53 (1H, dd, J = 0.6, 1.5 Hz). Anal. ($C_{10}H_7BrO_2S$) C, H, N.

Methyl 3-Iodoimidazo[1,2-*a*]pyridine-6-carboxylate (25b). To a solution of **24b** (8.81 g, 50.0 mmol) in acetonitrile (500 mL) at 0 °C was added NIS (12.4 g, 55.0 mmol), and the mixture was stirred at 80 °C for 5 h. The mixture was cooled to room temperature, and precipitated solid was filtered to give **25b** (11.0 g, 73%) as a pale-yellow solid. ^{1}H NMR ($CDCl_3$) δ 4.01 (3H, s), 7.69 (1H, d, J = 10.0 Hz), 7.80 (1H, s), 7.85 (1H, dd, J = 2.5, 10.0 Hz), 8.91 (1H, s).

Ethyl 3-Bromofuro[3,2-*b*]pyridine-5-carboxylate (25c). Bromine (7.82 mL, 157 mmol) was added dropwise to a solution of **24c** (0.30 g, 1.57 mmol) in CH_2Cl_2 (5 mL), and the mixture was stirred at room temperature for 3.5 h. The mixture was concentrated, diluted with THF, cooled to 0 °C, and treated dropwise with 1 M KOH in EtOH solution. After stirring at room temperature for 5 min, the mixture was poured into water and extracted with EtOAc. The organic layer was washed with brine, dried over sodium sulfate, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/EtOAc = 17/3 to 1/1) to give **25c** (0.15 g, 36%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 1.48 (3H, t, J = 7.2 Hz), 4.51 (2H, q, J = 7.2 Hz), 7.89 (1H, d, J = 8.7 Hz), 8.00 (1H, s), 8.22 (1H, d, J = 8.7 Hz).

Ethyl 3-Bromofuro[2,3-*b*]pyridine-5-carboxylate (25d). The compound **25d** was prepared in a manner similar to that described for **25c** in 30% yield as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 1.45 (3H, t, J = 7.2 Hz), 4.47 (2H, q, J = 7.2 Hz), 7.83 (1H, s), 8.56 (1H, d, J = 2.1 Hz), 9.06 (1H, d, J = 2.1 Hz). Anal. ($C_{10}H_8BrNO_3$) C, H, N.

The following compounds **26a**−**d** were prepared in a similar manner to that described for **2**.

Methyl 3-[4-(Methylsulfanyl)phenyl]-1-benzothiophene-5-carboxylate (26a). Yield 76%, colorless solid. ^{1}H NMR ($CDCl_3$) δ 2.56 (3H, s), 3.94 (3H, s), 7.38−7.42 (2H, m), 7.44 (1H, s), 7.49−7.53 (2H, m), 7.95 (1H, dd, J = 0.6, 8.5 Hz), 8.05 (1H, dd, J = 1.5, 8.5 Hz), 8.56 (1H, dd, J = 0.6, 1.5 Hz). Anal. ($C_{17}H_{14}O_2S_2$) C, H, N.

Methyl 3-[4-(Methylsulfanyl)phenyl]imidazo[1,2-*a*]pyridine-6-carboxylate (26b). Yield 42%, colorless solid. ^{1}H NMR ($CDCl_3$) δ 2.57 (3H, s), 3.94 (3H, s), 7.41−7.50 (4H, m), 7.65−7.69 (1H, m), 7.74−7.77 (2H, m), 9.02 (1H, t, J = 1.2 Hz).

Ethyl 3-[4-(Methylsulfanyl)phenyl]furo[3,2-*b*]pyridine-5-carboxylate (26c). Yield 32%, colorless solid. ^{1}H NMR ($CDCl_3$) δ 1.48 (3H, t, J = 7.2 Hz), 2.54 (3H, s), 4.51 (2H, q, J = 7.2 Hz), 7.38 (2H, d, J = 8.7 Hz), 7.88 (1H, d, J = 8.7 Hz), 8.09 (2H, d, J = 8.7 Hz), 8.18 (1H, d, J = 8.7 Hz), 8.21 (1H, s). Anal. ($C_{17}H_{15}NO_3S$) C, H, N.

Ethyl 3-[4-(Methylsulfanyl)phenyl]furo[2,3-*b*]pyridine-5-carboxylate (26d). Yield 95%, colorless solid. ^{1}H NMR ($CDCl_3$) δ 1.44 (3H, t, J = 7.2 Hz), 2.55 (3H, s), 4.46 (2H, q, J = 7.2 Hz), 7.39 (2H, d, J = 8.7 Hz), 7.58 (2H, d, J = 8.7 Hz), 7.94 (1H, s), 8.79 (1H, d, J = 1.9 Hz), 9.07 (1H, d, J = 2.3 Hz).

The following compounds **27a**−**d** were prepared in a similar manner to that described for **11**.

3-[4-(Methylsulfanyl)phenyl]-1-benzothiophene-5-carbohydrazide (27a). Yield 95%, colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.56 (3H, s), 4.51 (2H, brs), 7.41−7.46 (2H, m), 7.59−7.63 (2H, m), 7.85 (1H, dd, J = 1.5, 8.5 Hz), 7.89 (1H, s), 8.14 (1H, d, J = 8.5 Hz), 8.31 (1H, d, J = 1.5 Hz), 9.91 (1H, brs). Anal. ($C_{16}H_{14}N_2OS_2$) C, H, N.

3-[4-(Methylsulfanyl)phenyl]imidazo[1,2-*a*]pyridine-6-carbohydrazide (27b). Yield 76%, colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.56 (3H, s), 4.52 (2H, s), 7.45−7.48 (2H, d, J = 8.7 Hz), 7.64−7.72 (4H, m), 7.81 (1H, s), 8.90 (1H, s), 9.56 (1H, s).

3-[4-(Methylsulfanyl)phenyl]furo[3,2-*b*]pyridine-5-carbohydrazide (27c). Yield 82%, colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.54 (3H, s), 4.64 (2H, d, J = 4.3 Hz), 7.38 (2H, d, J = 8.5 Hz), 8.07 (1H, d, J = 8.7 Hz), 8.23 (1H, d, J = 8.7 Hz), 8.32 (2H, d, J = 8.5 Hz), 8.97 (1H, s), 9.93 (1H, brs). Anal. ($C_{15}H_{13}N_3O_2S$) C, H, N.

3-[4-(Methylsulfanyl)phenyl]furo[2,3-*b*]pyridine-5-carbohydrazide (27d). Yield 49%, colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.54 (3H, s), 4.60 (2H, brs), 7.43 (2H, d, J = 8.5 Hz), 7.78 (2H, d, J = 8.5 Hz), 8.63 (1H, s), 8.76−8.84 (2H, m), 10.06 (1H, brs).

2-Methyl-5-{3-[4-(methylsulfanyl)phenyl]-1-benzothiophen-5-yl}-1,3,4-oxadiazole (28a). The compound **28a** was prepared in a manner similar to that described for **7** in 93% as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 2.57 (3H, s), 2.63 (3H, s), 7.39−7.43 (2H, m), 7.47 (1H, s), 7.50−7.54 (2H, m), 8.02 (1H, dd, J = 0.8, 8.5 Hz), 8.07 (1H, dd, J = 1.5, 8.5 Hz), 8.49 (1H, dd, J = 0.8, 1.5 Hz). Anal. ($C_{18}H_{14}N_2OS_2$) C, H, N.

6-(5-Methyl-1,3,4-oxadiazol-2-yl)-3-[4-(methylsulfinyl)phenyl]imidazo[1,2-*a*]pyridine (28b). To a solution of **27b** (505 mg, 1.69 mmol) in DMA (4 mL) was added acetyl chloride (0.132 mL, 1.86 mmol), and the mixture was stirred at room temperature for 1.5 h. The reaction mixture was diluted with EtOAc and precipitated white solid was collected to give *N*′-acetyl-3-[4-(methylsulfanyl)phenyl]imidazo[1,2-*a*]pyridine-6-carbohydrazide (425 mg, 74%). A mixture of *N*′-acetyl-3-[4-(methylsulfanyl)phenyl]imidazo[1,2-*a*]pyridine-6-carbohydrazide (425 mg, 1.25 mmol) and TsCl (714 mg, 3.74 mmol) in pyridine (5 mL) was stirred at 100 °C for 1 day under argon atmosphere. After cooled to room temperature, the mixture was diluted with AcOEt. The organic layer was washed with saturated aqueous sodium hydrogen carbonate and brine, dried over magnesium sulfate, and concentrated in vacuo. The residue was purified by basic silica gel column chromatography (hexane/EtOAc = 4/1 to 1/1), and the product was recrystallized from hexane/chloroform to give **28b** (160 mg, 40%) as a yellow solid.

^{1}H NMR ($CDCl_3$) δ 2.57 (3H, s), 2.63 (3H, s), 7.42−7.52 (4H, m), 7.75−7.83 (3H, m), 8.95 (1H,s). Anal. ($C_{17}H_{14}N_4OS$) C, H, N.

5-(5-Methyl-1,3,4-oxadiazol-2-yl)-3-[4-(methylsulfanyl)phenyl]furo[3,2-*b*]pyridine (28c). To asolution of **27c** (0.20 g, 0.68 mmol and triethyl orthoacetate (0.27 mL, 1.49 mmol) was added *n*-BuOH (5 mL), and the mixture was stirred refluxed for 1.5 h. To the mixture was added DBU (0.10 mL, 0.68 mmol), and the mixture was refluxed overnight. The reaction mixture was cooled to room temperature and precipitated solid was recrystallized from hexane/EtOAc to give **28c** (0.15 g, 67%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 2.55 (3H, s), 2.71 (3H, s), 7.40 (2H, d, J = 8.5 Hz), 7.95 (1H, d, J = 8.7 Hz), 8.06 (2H, d, J = 8.5 Hz), 8.21 (1H, s), 8.28 (1H, d, J = 8.7 Hz). Anal. ($C_{17}H_{13}N_3O_2S$) C, H, N.

5-(5-Methyl-1,3,4-oxadiazol-2-yl)-3-[4-(methylsulfanyl)phenyl]furo[2,3-*b*]pyridine (28d). The compound **28d** was prepared in a manner similar to that described for **7** in 58% as a colorless solid.

^{1}H NMR (DMSO-d_6) δ 2.55 (3H, s), 2.63 (3H, s), 7.45 (2H, d, J = 8.5 Hz), 7.77 (2H, d, J = 8.5 Hz), 8.71 (1H, s), 8.82 (1H, d, J = 2.1 Hz), 8.98 (1H, d, J = 2.1 Hz).

The following compounds **29a**−**d** were prepared in a similar manner to that described for **9a**.

2-Methyl-5-{3-[4-(methylsulfinyl)phenyl]-1-benzothiophen-5-yl}-1,3,4-oxadiazole (29a). Yield 86%, colorless solid. ^{1}H NMR ($CDCl_3$) δ 2.64 (3H, s), 2.84 (3H, s), 7.58 (1H, s), 7.74−7.78 (2H, m), 7.81−7.85 (2H, m), 8.05 (1H, dd, J = 0.8, 8.5 Hz), 8.08 (1H, dd, J = 1.5, 8.5 Hz), 8.50 (1H, dd, J = 0.8, 1.5 Hz). Anal. ($C_{18}H_{14}N_2O_2S_2$) C, H, N.

6-(5-Methyl-1,3,4-oxadiazol-2-yl)-3-[4-(methylsulfinyl)phenyl]imidazo[1,2-*a*]pyridine (29b). Yield 82%, colorless solid. ^{1}H NMR ($CDCl_3$) δ 2.65 (3H, s), 2.84 (3H, s), 7.75−7.81 (2H, m), 7.83−7.89 (5H, m), 9.02 (1H, t, J = 1.5 Hz). Anal. ($C_{17}H_{14}N_4O_2S\cdot H_2O$) C, H, N.

5-(5-Methyl-1,3,4-oxadiazol-2-yl)-3-[4-(methylsulfinyl)phenyl]furo[3,2-*b*]pyridine (29c). Yield 41%, colorless solid. ^{1}H NMR ($CDCl_3$) δ 2.71 (3H, s), 2.78 (3H, s), 7.81 (2H, d, J = 8.7 Hz), 8.00 (1H, d, J = 8.7 Hz), 8.27−8.35 (4H, m). Anal. ($C_{17}H_{13}N_3O_3S$) C, H, N.

5-(5-Methyl-1,3,4-oxadiazol-2-yl)-3-[4-(methylsulfinyl)phenyl]furo[2,3-*b*]pyridine (29d). Yield 41%, colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.64 (3H, s), 2.81 (3H, s), 7.82−7.91 (2H, m), 7.98−8.08 (2H, m), 8.83 (1H, s), 8.89 (1H, d, J = 2.1 Hz), 9.01 (1H, d, J = 1.9 Hz). HPLC purity: 96%

***N*′-Acetyl-3-bromofuro[2,3-*c*]pyridine-5-carbohydrazide (31).** To a solution of **30** (1.58 g, 6.53 mmol) and acetohydrazine (611 mg, 7.83 mmol) in DMF (10 mL) were added EDC (1.50 g, 7.83 mmol) and HOBt (1.06 g, 7.83 mmol), and the mixture was stirred at room temperature for 2 days. The reaction mixture was added into water, and precipitated solid was collected to give **31** (0.55 g, 28%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 1.93 (3H, s), 8.20 (1H, d, J = 0.9 Hz), 8.70 (1H, s), 9.12 (1H, d, J = 0.9 Hz), 10.06 (1H, s), 10.46 (1H, s).

3-Bromo-5-(5-methyl-1,3,4-oxadiazol-2-yl)furo[2,3-*c*]pyridine (32). The compound **32** was prepared in a manner similar to that described for **22** in 42% as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 2.69 (3H, s), 7.90 (1H, s), 8.51 (1H, d, J = 0.9 Hz), 9.00 (1H, d, J = 0.9 Hz).

5-(5-Methyl-1,3,4-oxadiazol-2-yl)-3-[4-(methylsulfanyl)phenyl]furo[2,3-*c*]pyridine (33). The compound **33** was prepared in a manner similar to that described for **2** in 52% as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.55 (3H, s), 2.63 (3H, s), 7.46 (2H, d, J = 8.3 Hz), 7.76 (2H, d, J = 8.3 Hz), 8.61 (1H, s), 8.80 (1H, s), 9.21 (1H, s).

5-(5-Methyl-1,3,4-oxadiazol-2-yl)-3-[4-(methylsulfinyl)phenyl]furo[2,3-*c*]pyridine (34). The compound **34** was prepared in a manner similar to that described for **9a** in 67% as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.64 (3H, s), 2.82 (3H, s), 7.88 (2H, d, J = 8.4 Hz), 8.03 (2H, d, J = 8.4 Hz), 8.66 (1H, d, J = 0.9 Hz), 8.92 (1H, s), 9.24 (1H, d, J = 0.9 Hz). Anal. ($C_{17}H_{13}N_3O_3S$) C, H, N.

4-(2,4-Dimethoxybenzyl)-3-methyl-5-{3-[4-(methylsulfinylphenyl]-1-benzofuran-5-yl}-4*H*-1,2,4-triazole (35). To a solution of **9b** (0.80 g, 2.36 mmol), 2,4-dimethoxybenzylamine (5.34 mL, 35.5 mmol), and *p*-TsOH (46 mg, 0.24 mmol) in xylene (10 mL) was stirred overnight at 150 °C. The reaction mixture was diluted with water and extracted with EtOAc. The organic layer was washed with brine, dried over sodium sulfate, and concentrated in vacuo. The residue was purified by basic silica gel (hexane/EtOAc = 1/1 to 0/1), and the product was recrystallized from hexane/EtOAc to give **35** (0.51 g, 44%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.34 (3 H, s), 2.80 (3 H, s), 3.65 (3 H, s), 3.75 (3 H, s), 5.08 (2 H, s), 6.46 (1 H, dd, J = 1.9 8.3 Hz), 6.51 (1 H, d, J = 8.3 Hz), 6.60 (1 H, d, J = 1.9 Hz), 7.62 (1 H, dd, J = 1.5, 8.3 Hz), 7.66−7.75 (4 H, m), 7.84 (1 H, d, J = 8.3 Hz), 7.87 (1 H, d, J = 1.5 Hz), 8.59 (1 H, s).

3-Methyl-5-{3-[4-(methylsulfinyl)phenyl]-1-benzofuran-5-yl}-4*H*-1,2,4-triazole (36). A mixture of **35** (0.405 g, 0.83 mmol) in TFA (10 mL) was stirred overnight at room temperature. The mixture was concentrated, and saturated aqueous sodium hydrogen carbonate was added and extracted with EtOAc. The organic layer was washed with brine, dried over magnesium sulfate, and concentrated in vacuo. The residue was purified by basic silica gel column chromatography (EtOAc/MeOH = 1/0 to 97/3), and the product was recrystallized from acetone to give **36** (0.11 g, 39%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.40 (3 H, s), 2.82 (3 H, s), 7.78 (1 H, d, J = 8.7 Hz), 7.87 (2 H, d, J = 8.4 Hz), 7.97 (2 H, d, J = 8.4 Hz), 8.06 (1 H, dd, J = 1.7, 8.7 Hz), 8.48−8.50 (1 H, m), 8.54 (1 H, s), 13.77 (1 H, brs). Anal. ($C_{18}H_{15}N_3O_2S$) C, H, N.

***N*′-Acetyl-3-[4-(methylsulfanyl)phenyl]-1-benzofuran-5-carbohydrazide (37).** To a solution of **11** (1.0 g, 3.35 mmol) and AcOH (0.29 mL, 5.03 mmol) in DMF (10 mL) were added EDC (0.86 g, 5.03 mmol) and HOBt (0.68 g, 5.03 mmol), and the reaction mixture was stirred at room temperature for 15 h. The mixture was diluted with water and extracted with EtOAc. The organic layer was washed with water, dried over magnesium sulfate, and concentrated in vacuo. The residue was crystallized from hexane/Et_2O to give **37** (0.85 g, 75%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 1.92 (3H, s), 2.53 (3H, s), 7.40 (2H, d, J = 8.4 Hz), 7.70−7.80 (3H, m), 7.90 (1H, d, J = 8.7 Hz), 8.41 (1H, s), 8.45 (1H, s), 9.88 (1H, brs), 10.41 (1H, brs).

2-Methyl-5-{3-[4-(methylsulfanyl)phenyl]-1-benzofuran-5-yl}-1,3,4-thiadiazole (38). The mixture of compound **37** (0.76 g, 2.23 mmol), Lawesson's reagent (1.81 g, 4.46 mmol), and toluene (100 mL) was stirred at 80 °C for 1 h and purified by column chromatography (basic silica gel, EtOAc). The crude product was crystallized from EtOAc to give **38** (0.41 g, 54%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.54 (3H, s), 2.78 (3H, s), 7.43 (2H, d, J = 8.1 Hz), 7.72 (2H, d, J = 8.1 Hz), 7.84 (1H, d, J = 8.4 Hz), 7.95 (1H, dd, J = 2.1, 9.0 Hz), 8.37 (1H, s), 8.49 (1H, s). Anal. ($C_{18}H_{14}N_2OS_2$) C, H, N.

2-Methyl-5-{3-[4-(methylsulfinyl)phenyl]-1-benzofuran-5-yl}-1,3,4-thiadiazole (39). Compound **39** was prepared in a manner similar to that described for **9a** in 93% as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.79 (3H, s), 2.81 (3H, s), 7.80−7.90 (3H, m), 7.95−8.05 (3H, m), 8.42 (1H, d, J = 1.8 Hz), 8.61 (1H, s). Anal. ($C_{18}H_{14}N_2O_2S_2$) C, H, N.

3-Methyl-5-{3-[4-(methylsulfanyl)phenyl]-1-benzofuran-5-yl}-1,2,4-oxadiazole (40). To a solution of acetoamidoxime (0.19 g, 2.58 mmol) in THF (15 mL) was added a 60% suspension of sodium hydride in mineral oil (103 mg, 2.58 mmol). The reaction mixture was stirred at room temperature for 15 min and at 60 °C for 30 min. Then to the mixture was added **10** (0.70 g, 2.35 mmol), and the mixture was stirred at 60 °C for 2 h. The reaction mixture was diluted with water and extracted with EtOAc. The organic layer was washed with water and dried over magnesium sulfate and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/EtOAc = 3/1 to 2/1), and the product was recrystallized from EtOAc to give **40** (0.51 g, 67%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 2.43 (3H, s), 3.32 (3H, s), 7.45 (2H, d, J = 8.4 Hz), 7.72 (2H, d, J = 8.4 Hz), 7.92 (1H, d, J = 8.7 Hz), 8.10−8.20 (1H, m), 8.55−8.60 (2H, m). Anal. ($C_{18}H_{14}N_2O_2S$) C, H, N.

3-Methyl-5-{3-[4-(methylsulfinyl)phenyl]-1-benzofuran-5-yl}-1,2,4-oxadiazole (41). The compound **41** was prepared in a manner similar to that described for **9a** in 91% as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.44 (3H, s), 2.82 (3H, s), 7.87 (2H, d, J = 8.4 Hz), 7.95 (2H, d, J = 9.0 Hz), 7.99 (1H, d, J = 8.4 Hz), 8.14 (1H, d, J = 8.4 Hz), 8.58 (1H, s), 8.67 (1H, s). Anal. ($C_{18}H_{14}N_2O_3S$) C, H, N.

3-[4-(Methylsulfanyl)phenyl]-1-benzofuran-5-carboxylic Acid (42). To a solution of **10** (3.3 g, 10.2 mmol) in MeOH (20 mL) and THF (10 mL) was added 1 M NaOH aqueous solution (20.5 mL, 20.5 mmol), and the mixture was stirred at room temperature for 17 h. Then 1 M HCl aqueous solution (22 mL) was added and concentrated in vacuo. The residue was diluted with water and precipitated solid was filtered to give **42** (2.8 g, 96%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 2.54 (3H, s), 7.43 (2H, d, J = 8.1 Hz), 7.68 (2H, d, J = 8.1 Hz), 7.76 (1H, d, J = 9.0 Hz), 7.99 (1H, d, J = 9.0 Hz), 8.44 (1H, s), 8.48 (1H, s), 12.99 (1H, brs). Anal. ($C_{16}H_{12}O_3S$) C, H, N.

3-[4-(Methylsulfanyl)phenyl]-1-benzofuran-5-carboxamide (43). The compound **43** was prepared in a manner similar to that described for **31** in 95% as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.54 (3H, s), 7.37 (1H, brs), 7.41 (2H, d, J = 8.7 Hz), 7.70−7.80 (3H, m), 7.92 (1H, d, J = 8.7 Hz), 8.13 (1H, brs), 8.41 (1H, s), 8.44 (1H, s). Anal. ($C_{16}H_{13}NO_2S$) C, H, N.

3-[4-(Methylsulfanyl)phenyl]-1-benzofuran-5-carbonitrile (44). To a solution of **43** (2.35 g, 8.29 mmol) in DMF (10 mL) was added dropwise thionyl chloride (1.21 mL, 16.6 mmol), and the mixture was stirred at room temperature for 5 h. The reaction mixture was quenched by the addition of saturated aqueous sodium hydrogen carbonate and extracted with EtOAc. The organic layer was washed with water, dried over magnesium sulfate, and concentrated in vacuo. This residue was purified by silica gel column chromatography (toluene), and the product was recrystallized from cold hexane/Et_2O to give **44** (1.67 g, 76%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.53 (3H, s), 7.38 (2H, d, J = 8.1 Hz), 7.73 (2H, d, J = 8.1 Hz), 7.80−8.00 (2H, m), 8.45 (1H, s), 8.58 (1H, s). Anal. ($C_{16}H_{11}NOS$) C, H, N.

5-Methyl-3-{3-[4-(methylsulfanyl)phenyl]-1-benzofuran-5-yl}-1,2,4-oxadiazole (45). A solution of **44** (1.32 g, 4.97 mmol), hydroxylamine hydrochloride (1.04 g, 14.9 mmol), and sodium hydrogen carbonate (1.25 g, 14.9 mmol) in MeOH (20 mL) was refluxed for 6 h and concentrated. The residue was added to water and precipitated solid was filtered and washed with water. A mixture of the solid and acetic anhydride (0.94 mL, 9.95 mmol) in dioxane (20 mL) was refluxed at 90 °C for 15 h. The mixture was added into saturated aqueous sodium hydrogen carbonate and extracted with EtOAc. The organic layer was washed with water and brine, dried over magnesium sulfate, and concentrated in vacuo. The residue was purified by silica gel column chromatography (toluene), and the product was recrystallized from acetone/Et_2O to give **45** (1.22 g, 76%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.54 (3H, s), 3.32 (3H, s), 7.44 (2H, d, J = 8.4 Hz), 7.69 (2H, d, J = 8.4 Hz), 7.85 (1H, d, J = 8.7 Hz), 8.03 (1H, d, J = 8.7 Hz), 8.44 (1H, s), 8.48 (1H, s). Anal. ($C_{18}H_{14}N_2O_2S$) C, H, N.

5-Methyl-3-{3-[4-(methylsulfinyl)phenyl]-1-benzofuran-5-yl}-1,2,4-oxadiazole (46). Compound **46** was prepared in a manner similar to that described for **9a** in 87% as a colorless solid. ^{1}H

NMR (DMSO-d_6) δ 2.69 (3H, s), 2.82 (3H, s), 7.80−7.95 (3H, m), 7.95 (2H, d, J = 8.4 Hz), 8.06 (1H, d, J = 8.7 Hz), 8.48 (1H, s), 8.60 (1H, s). Anal. ($C_{18}H_{14}N_2O_3S$) C, H, N.

3-Bromo-1-benzofuran-5-carbaldehyde (47). To a solution of **3** (1.0 g, 6.75 mmol) in PhCl (20 mL) were added NBS (1.44 g, 8.1 mmol) and AIBN (22 mg, 0.13 mmol), and the mixture was stirred at 80 °C for 1 h. The mixture was cooled, washed with saturated aqueous sodium bicarbonate, dried over magnesium sulfate, and concentrated in vacuo to give 1-benzofuran-5-carbaldehyde (0.82 g, 83%) as a pale-orange oil.

To a solution of 1-benzofuran-5-carbaldehyde (0.8 g, 5.47 mmol) in CH_2Cl_2 (5 mL) was added dropwise bromine (0.36 mL, 7.12 mmol), and the mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated in vacuo and diluted with EtOH (5 mL). Potassium hydroxide (0.68 g, 12.0 mmol) was added, and the mixture was stirred at 60 °C for 30 min and then diluted with water and extracted with EtOAc. The organic layer was washed with water, dried over magnesium sulfate, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/acetone = 20:1), and the product was recrystallized from cold hexane to give the **47** (0.19 g, 15%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 7.62 (1H, d, J = 8.7 Hz), 7.76 (1H, s), 7.94 (1H, dd, J = 1.8, 8.7 Hz), 8.10 (1H, d, J = 1.8 Hz), 10.10 (1H, s). Anal. ($C_9H_5BrO_2$) C, H, N.

1-(3-Bromo-1-benzofuran-5-yl)pentane-1,4-dione (48). A solution of **47** (225 mg, 0.10 mmol), methyl vinyl ketone (0.10 mL, 0.12 mmol), 3-ethyl-5-(2-hydroxyethyl)-4-methylthiazolium bromide (63 mg, 0.025 mmol), NEt_3 (0.21 mL, 0.15 mmol), and EtOH (5 mL) was stirred at 70 °C for 1 h. The mixture was diluted with water and extracted with EtOAc. The organic layer was washed with water, dried over magnesium sulfate, and concentrated in vacuo. This residue was purified by silica gel column chromatography (hexane/acetone = 3/1) to give the **48** (73 mg, 25%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 2.28 (3H, s), 2.93 (2H, t, J = 6.3 Hz), 3.37 (2H, t, J = 6.3 Hz), 7.54 (1H, d, J = 8.7 Hz), 7.72 (1H, s), 8.04 (1H, dd, J = 1.5, 8.4 Hz), 8.21 (1H, s).

3-(3-Bromo-1-benzofuran-5-yl)-6-methylpyridazine (49). To a solution of **48** (2.24 g, 7.60 mmol) in EtOH (20 mL) was added hydrazine hydrate (0.40 mL, 8.35 mmol), and the mixture was refluxed for 5 h. The reaction mixture was added to water and extracted with EtOAc. The organic layer was washed with water dried over magnesium sulfate and concentrated in vacuo. This residue was purified by silica gel column chromatography (hexane/acetone = 3/1 to 1/1), and the product was recrystallized from hexane/Et_2O to give **49** (0.51 g, 23%) as a colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.78 (3H, s), 7.40 (1H, d, J = 8.7 Hz), 7.61 (1H, d, J = 8.7 Hz), 7.71 (1H, s), 7.83 (1H, d, J = 8.7 Hz), 8.14 (1H, d, J = 8.7 Hz), 8.21 (1H, s). Anal. ($C_{13}H_9BrN_2O$) C, H, N.

3-Methyl-6-{3-[4-(methylsulfanyl)phenyl]-1-benzofuran-5-yl}pyridazine (50). The compound **50** was prepared in a manner similar to that described for **2** in 68% as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.54 (3H, s), 2.67 (3H, s), 7.43 (2H, d, J = 8.7 Hz), 7.66 (1H, d, J = 8.7 Hz), 7.76 (2H, d, J = 8.7 Hz), 7.82 (1H, d, J = 8.7 Hz), 8.17 (1H, d, J = 8.7 Hz), 8.26 (1H, d, J = 8.7 Hz), 8.45 (1H, s), 8.58 (1H, s).

3-Methyl-6-{3-[4-(methylsulfinyl)phenyl]-1-benzofuran-5-yl}pyridazine (51). Compound **51** was prepared in a manner similar to that described for **9a** in 87% as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.67 (3H, s), 2.81 (3H, s), 7.67 (1H, d, J = 9.0 Hz), 7.84 (2H, d, J = 8.4 Hz), 7.86 (1H, d, J = 8.4 Hz), 8.03 (2H, d, J = 8.1 Hz), 8.20 (1H, d, J = 9.0 Hz), 8.29 (1H, d, J = 8.4 Hz), 8.58 (1H, s), 8.62 (1H, s). Anal. ($C_{20}H_{16}N_2O_2S$) C, H, N.

Optical Resolution of 9b. Compound **9b** (1.0 g, 2.96 mmol) was separated by preparative HPLC (CHIRALPAK AS (0.50 cm × 50 cm), EtOH, 45 mL/min, UV254 nm) to afford (*R*)-**9b** (0.49 g, 49% yield) and (*S*)-**9b** (0.49 g, 49% yield). (*R*)-**9b**: $[\alpha]^{25}_D$ +86.9 (c 0.55, MeOH); t_R = 17.9 min (CHIRALPAK AS 4.6 mmID×250 mmL, EtOH, 0.4 mL/min); 99.9% ee. (*S*)-**9b**: $[\alpha]^{25}_D$ −89.9 (c 0.49, MeOH); t_R = 25.4 min (CHIRALPAK AS 4.6 mmID×250 mmL, EtOH, 0.4 mL/min); 99.9% ee.

Optical Resolution of 9c. Compound **9c** (530 mg, 1.50 mmol) was separated by preparative HPLC (CHIRALCEL OJ (0.50 cm × 50 cm), hexane/EtOH 1:1, 80 mL/min, UV254 nm) to afford (*R*)-**9c** (249 mg, 47% yield) and (*S*)-**9c** (245 mg, 46% yield). (*R*)-**9c**: $[\alpha]^{25}_D$ +127.2; t_R = 19.8 min (CHIRALCEL OJ 4.6 mmID×250 mmL, hexane/EtOH 1:1, 0.5 mL/min); 99.9% ee. (*S*)-**9c**: $[\alpha]^{25}_D$ −130.4; t_R = 30.5 min (CHIRALCEL OJ 4.6 mmID×250 mmL, hexane/EtOH 1:1, 0.5 mL/min); 99.9% ee.

X-ray Crystallographic Data. Data obtained for (*S*)-**9b** and (*S*)-**9c** are included in Supporting Information. X-ray crystallographic data for compounds (*S*)-**9b** and (*S*)-**9c** have been deposited with the Cambridge Crystallographic Data Center as CCDC 711975 and CCDC 711976, respectively. The crystallographic data can be obtained free of charge by writing to CCDC, 12 Union Road, CAMBRIDGE, CB2, IEZ, UK (fax (+44) 1223 336033, e-mail: deposit@ccdc.cam.ac.uk).

Expression, Purification, Crystallization, and Structure Determination. The gene for human GSK-3β (residues 48 to 433) was cloned into a pSXB100 baculovirus expression vector with an *n*-terminal 6-Histidine tag containing an rTEV protease cleavage site and expressed and purified essentially as described previously.[22] Briefly, the expressed protein was purified by immobilized metal-chelate affinity chromatography (IMAC), the eluted protein treated with rTEV protease to remove the tag, and subjected to a second round of IMAC purification followed by dialysis and further purification by cation exchange chromatography to obtain the singly phosphorylated protein species. Inhibitor in DMSO solution was added slowly, with stirring, to the dilute protein (~1 mg/mL) to a final concentration of 1 mM, and the enzyme inhibitor complex further concentrated to 10−15 mg/mL for crystallization experiments. Cocrystals of the complex are grown at 4 °C by mixing 50 nL of enzyme: inhibitor solution with 50 nL of precipitant solution containing 20% PEG 3350 and 0.15 M NaCl and are flash-frozen by direct immersion in liquid nitrogen using ethylene glycol as a cryoprotectant. X-ray diffraction data were collected at the Advanced Light Source in Berkeley, CA, on Beamline 5.0.3 at a wavelength of 1.0 Å. Diffraction data for the cocrystal belong to the orthorhombic space group $C222_1$ with unit cell dimensions 85.3 Å × 104.1 Å × 110.0 Å and extend to 2.4 Å resolution with an R_{merge} of 0.066. The structures were solved by molecular replacement with AMoRe[33] using the previously reported structure of GSK-3β (PDB entry 3F88) and refined with REFMAC within the CCP4 suite of programs.[34] The final refined crystallographic statistics for the structure are, R = 22.3 (R_{free}, 28.9), with root-mean-square deviations (rmsd) in the bond lengths of 0.012 Å, and in the bond lengths of 1.44°. The structure has been deposited with the RCSB structure database with accession codes 3GB2.

GSK-3β and CDK5 Kinase Assay. The human GSK-3β expressed as N-terminal FLAG-tagged proteins using baculovirus expression system (Takeda Pharmaceutical Company Ltd., Osaka, Japan). The human p35/cyclin dependent kinase 5 (CDK5) was purchased from Millipore Corp. (Bedford, MA), which was expressed as N-terminal GST fusion protein using baculovirus expression system. The kinase assay was performed in a reaction mixture that contained 25 mM HEPES, pH 7.5, 10 mM magnesium acetate, 1 mM dithiothreitol, and 0.01% BSA and serially diluted test compounds. The assay was conducted in a 96-well plate assay format. The end amount of enzyme was 20 ng/well for CDK5 or 40 ng/well for GSK-3β. The end amount of substrate was 1 ng/well of CDK5 substrate peptide for CDK5 (Calbiochem, La Jolla, CA) for CDK5 or 100 ng/well of GSK-3β substrate peptide (Millipore Corp.) for GSK-3β.

All the kinase reactions were started by addition of the ATP-solution (final 500 nM) and were incubated for 90 min at 37 °C for GSK-3β or for 45 min at room temperature for CDK5. The

reactions were terminated by the Kinase-Glo reagent contained EDTA (50 μL/well, Promega Corp., Madison, WI). Ten minutes after addition of the Kinase-Glo reagent, the luminescence was measured on a Wallac ARVO 1420 (PerkinElmer, Shelton, CT). The reaction window was calculated from the difference of the average signals obtained from the control (5% DMSO) and the background wells. The inhibition with compounds was expressed by the inhibitor concentration that produced 50% inhibition (IC_{50}) of the activity without compound. The IC_{50} values were obtained by linear regression analysis with a GraphPad Prism (version 3.02 for Windows, GraphPad software, Inc. San Diego, CA). The best-fit lines were obtained by analyzing the logistic fitting equation.

Serine/Threonine Kinase Profiling by IC_{50} Measurement. Assays for 10 serine/threonine kinases using radio labeled [γ-^{33}P] ATP (GE Healthcare, Piscataway, NJ) were performed in 96-well plates. Mitogen-activated protein kinase p38 alpha (p38α), protein kinase C theta (PKCθ) and Jun N-terminal kinase 1 (JNK1) were expressed as N-terminal FLAG tagged protein using baculovirus expression system. I kappa B kinase β (IKKβ) and MEK kinase 1 (MEKK1) were expressed as C-terminal FLAG tagged protein using baculovirus expression system. Casein kinase 1 delta (CK1δ) was expressed as N-terminal GST fusion protein using *Escherichia coli* expression system. Checkpoint kinase 1 (CHK1) was expressed as N-terminal GST fusion protein using baculovirus expression system. CDK1/CycB and CDK2/CycA were expressed as C-terminal 6xHis-tagged CDK1 or CDK2, and N-terminal GST-tagged cyclin B or cyclin A protein using the baculovirus expression system. The reaction conditions were optimized for each kinase: p38α (100 ng/well of enzyme, 1 μg/well of myelin basic protein (MBP) (Wako Pure Chemical Industries, Osaka, Japan), 0.1 μCi/well of [γ-^{33}P] ATP, 60 min reaction at 30 °C); MEKK1 (25 ng/well of enzyme, 1 μg/well of MBP, 0.1 μCi/well of [γ-^{33}P] ATP, 60 min reaction at 30 °C); PKCθ (25 ng/well of enzyme, 2 μg/well of MBP, 0.1 μCi/well of [γ-^{33}P] ATP, 60 min reaction at 30 °C); JNK1 (10 ng/well of enzyme, 1 μg/well of c-Jun, 0.1 μCi/well of [γ-^{33}P] ATP, 60 min reaction at 30 °C); IKKβ (20 ng/well of enzyme, 1 μg/well of IκBα, 0.1 μCi/well of [γ-^{33}P] ATP, reaction at room temperature); CDK1/CycB (4.2 ng/well of enzyme, 1 μg/well of Histone H1 (Calbiochem), 0.2 μCi/well of [γ-^{33}P] ATP, 20 min reaction at room temperature); CDK2/CycA (1.8 mUnits/well of enzyme, 1 μg/well of Histone H1, 0.2 μCi/well of [γ-^{33}P] ATP, 20 min reaction at room temperature); CK1δ (120 ng/well of enzyme, 2.4 μM of CK1tide (Millipore Corp.), 0.2 μCi/well of [γ-^{33}P] ATP, 20 min reaction at room temperature); CHK1 (30 ng/well of enzyme, 25 μM of CHKtide (Millipore Corp.), 0.2 μCi/well of [γ-^{33}P] ATP, 10 min reaction at room temperature). Except for the PKCθ, enzyme reactions were performed in 25 mM HEPES, pH 7.5, 10 mM magnesium acetate, 1 mM dithiothreitol, and 500 nM ATP containing optimized concentration of enzyme, substrate, and radiolabeled ATP as described above in a total volume of 50 μL. For the PKCθ, enzyme reactions were performed in 25 mM HEPES, pH 7.5, 10 mM magnesium acetate, 1 mM dithiothreitol, lipid activator (Millipore Corp.).

Prior to the kinase reaction, compound and enzyme were incubated for 5 min at reaction temperature as described above. The kinase reactions were initiated by the addition of ATP. After the reaction period as described above, the reactions were terminated by the addition of 10% trichloroacetic acid (final concentration). The [γ-^{33}P]-phosphorylated proteins were filtrated in Harvest Plate (Millipore Corp.) with a Cell Harvester (PerkinElmer) and then free of [γ-^{33}P] ATP was washed out with 3% phosphoric acid. The plates were dried, followed by the addition of 40 μL of MicroScint0 (PerkinElmer). The radioactivity was counted by a TopCount scintillation counter (PerkinElmer).

Tyrosine Kinase Profiling by IC_{50} Measurement. The cytoplasmic domain of vascular endothelial growth factor receptor 2 (VEGFR2) was expressed as N-terminal FLAG-tagged proteins using the baculovirus expression system. The cytoplasmic domains of v-erb-a erythroblastic leukemia viral oncogene homologue 2 (ERBB2) and epidermal growth factor receptor (EGFR) were expressed as N-terminal peptide (DYKDDDD)-tagged protein using baculovirus expression system. These expressed kinase proteins were purified by using anti-FLAG M2 affinity gel (Sigma-Aldrich, St. Louis, MO). Fibroblast growth factor receptor 3 (FGFR3), platelet-derived growth factor receptor alpha (PDGFRα), PDGFRβ, TIE2, c-Met, c-Kit, Src, insulin receptor (IR), and lymphocyte-specific protein tyrosine kinase (Lck) were purchased from Millipore Corp. Assays for 10 tyrosine kinases except ERBB2 and EGFR using antiphosphotyrosine antibody were performed in 384-well plates using the Alphascreen system (PerkinElmer) at room temperature. Enzyme reactions were performed in 50 mM Tris-HCl, pH 7.5, 5 mM $MnCl_2$, 5 mM $MgCl_2$, 0.01% Tween-20, 2 mM dithiothreitol, and 0.1 μg/mL biotinylated poly-GluTyr (4:1) containing optimized concentration of enzyme, ATP as described below. Prior to the kinase reaction, compound and enzyme were incubated for 5 min at room temperature. The reactions were initiated by the addition of ATP. After the reaction period as described below at room temperature, the reactions were stopped by the addition of 25 μL of 100 mM EDTA, 10 μg/mL Alphascreen streptavidine donor beads, and 10 μg/mL acceptor beads described below in 62.5 mM HEPES, pH 7.4, 250 mM NaCl, and 0.1% BSA. The plates were incubated in the dark for more than 12 h and then read by EnVision 2102 Multilabel Reader (PerkinElmer). The well containing substrate and enzyme without compound was used as total reaction control. The reaction conditions for these 10 kinases were optimized for each kinase: VEGFR2 (19 ng/mL of enzyme, 10 μM ATP, 10 min reaction, PY-100 conjugated acceptor beads (PY-100)); FGFR3 (20 ng/mL of enzyme, 20 μM ATP, 10 min reaction, PY-100); PDGFRα (50 ng/mL of enzyme, 10 μM ATP, 30 min reaction, PT66 conjugated acceptor beads (PT66)); PDGFRβ (50 ng/mL of enzyme, 20 μM ATP, 60 min reaction, PT66); TIE2 (20 ng/mL of enzyme, 2 μM ATP, 10 min reaction, PT66); c-Met (1 ng/mL of enzyme, 2 μM ATP, 10 min reaction, PT66); c-Kit (10 ng/mL of enzyme, 20 μM ATP, 20 min reaction, PT66); Src (0.33 ng/mL of enzyme, 2 μM ATP, 10 min reaction, PY-100); IR (100 ng/mL of enzyme, 10 μM ATP, 60 min reaction, PT66); Lck (100 ng/mL of enzyme, 2 μM ATP, 30 min reaction, PY-100). Assays for ERBB2 and EGFR kinase using radio labeled [γ-^{32}P] ATP (GE Healthcare) were performed in 96-well plates. ERBB2 and EGFR kinase reactions were performed in 50 mM Tris-HCl, pH 7.5, 5 mM $MnCl_2$, 0.01% Tween-20, and 2 mM dithiothreitol containing 0.9 μCi of [γ-^{32}P] ATP per reaction, 50 μM ATP, 5 μg/mL poly-Glu-Tyr (4:1), 0.1% DMSO, and 0.25 μg/mL of ERBB2 or EGFR cytoplasmic domains in a total volume of 50 μL. Prior to the kinase reaction, compound and enzyme were incubated for 5 min at room temperature. The kinase reactions were initiated by the addition of ATP. After the kinase reaction for 10 min (ERBB2) and 5 min (EGFR) at room temperature, the reactions were terminated by the addition of 10% trichloroacetic acid (final concentration). The [γ-^{32}P]-phosphorylated proteins were filtrated in Harvest plate (Millipore Corp.) with a Cell harvester (PerkinElmer) and washed free of [γ-^{32}P] ATP with 3% phosphoric acid. The plate was dried, followed by the addition of 25 μL of MicroScint0 (PerkinElmer). The radioactivity was counted by a Topcount scintillation counter (PerkinElmer).

In Vivo Evaluation in Cold Water Stress Model. Compounds were suspended in 0.5% methylcellulose and were administered orally in a volume of 3 mL/kg of body weight 30 min before the CWS. Mice ($N = 5$) were immersed up to the neck in cold water (1−2 °C) for 4 min and then were returned to individual cages. Mice were sacrificed by decapitation 10 min after CWS, the brains were immediately removed, and tissues were dissected.

Antibodies. The following antibodies were used for immunoblotting at appropriate concentrations as recommended by the

manufacturers. Rabbit polyclonal antibodies recognizing phospho-Thr205 tau was purchased from Biosource International (Camarillo, CA). A rabbit polyclonal antibody recognizing total tau, tau Ab-3, was purchased from Lab Vision Corporation (Fremont, CA).

Animals. Male C57BL/6Njcl mice, 8−12 weeks of age, (Clea Japan, Tokyo, Japan) were used for the CWS model. All animals used in this study were housed in groups and allowed free access to food and water and were cared for in accordance with the Principles and Guidelines on Animal Experimentation of the Pharmaceutical Research Division of Takeda Pharmaceutical Company Ltd.

Preparation of Hippocampal Protein Extract. Hippocampi were immediately homogenized in ice cold radioimmunoprecipitation assay (RIPA) buffer (50 mM Tris-HCl (pH 7.6), 5 mM ethylenediamine tetra acetic acid (EDTA), 1 mM ethylene glycol tetraacetic acid (EGTA), 30 mM NaF, 5 mM sodium diphosphate, 2 μM pepstatin A, 100 mM NaCl, 1% NP-40, 0.25% sodium deoxycholate, 1 μM microcystin LR, 40 μM leupeptin, 100 μM 4-(2-aminoethyl)benzenesulfonyl fluoride (ABSF), 2 mM sodium orthovanadate, 1 μM MG115), and centrifuged at 20000*g* for 10 min at 4 °C. The supernatants were transferred to fresh tubes. The protein concentrations were determined by BCA protein assay (PIERCE, Rockford, IL).

SDS-PAGE and Western Blotting. Samples containing 3−10 μg of protein were separated on a 10% SDS-polyacrylamide gel and transferred to a polyvinylidene fluoride (PVDF) membrane. The membrane was washed once with 0.1% Tween-20 containing Tris-buffered saline (TBS-T) for 15 min before being treated with Block-Ace (DS Pharma Biomedical, Tokyo, Japan) for 45 min and then probed with primary antibody in TBS-T containing 3% bovine serum albumin (BSA). The membrane was washed 3 times with TBS-T and then incubated with the antirabbit IgG horseradish peroxidase-linked species-specific F(ab′)2 fragment (GE Healthcare, Piscataway, NJ) in TBS-T for 1 h. After washing 3 times with TBS-T, the blot was developed with ImmunoStar Reagents (Wako Pure Chemical Industries). The images obtained with a CCD camera (LAS-1000plus Luminescent Image Analyzer; Fuji Film, Tokyo, Japan) were quantified by densitometry (Image Gauge version 3.46; Fuji Film).

Statistical Analyses. Statistical analysis was performed by statistical analysis software (SAS preclinical package, version 5.0; SAS Institute, Inc., Cary, NC). Values were expressed as mean ± SEM and statistically analyzed using an unpaired Student's *t* test. In the CWS model, the inhibitory rate of each stressed group was calculated by defining the mean value of the normal group as 0% and that of the CWS-treated control group as 100%.

Pharmacokinetic Studies. Compounds (*S*)-**9b** and (*S*)-**9c** were administered to nonfasted Crl:CD(SD)IGS rats (male, 8 weeks old, $n = 3$) intravenously (1 mg/kg, DMA/1, 3-butandiol) and orally (3 mg/kg, 0.5% methylcellulose suspension). At 5, 10, 15, and 30 min and 1, 2, 4, 8, 24 h after intravenous administration or at 15 and 30 min and 1, 2, 4, 8, 24 h after oral administration, blood samples were collected from tail vein. The blood samples were centrifuged to obtain the plasma fraction. The plasma samples were deproteinized with acetonitrile containing an internal standard. After centrifugation, the supernatant obtained was diluted with 0.01 mol/L ammonium acetate and centrifuged again. The compound concentration in the supernatant was measured by LC/MS/MS with an API4000 triple quadrupole mass spectrometer (MDS Sciex). The mass spectrometer was equipped with a turbo ionspray source and operated in positive ion mode. The HPLC conditions were as follows: column, Chiralcel OJ-RH (2.1 mm × 150 mm); mobile phase, (A) 0.01 mol/L ammonium acetate/(B) acetonitrile = 6/4; gradient condition, 0−7 min: 40%B; 7−7.2 min: 40−85% B; 7.2−13.5 min: 85% B; 13.5−14 min: 85−40% B; 14−30 min: 40% B; flow rate, 0.2 mL/min; column temperature, 40 °C.

Brain and Plasma Concentration in Mice. Compounds (*S*)-**9b** and (*S*)-**9c** were administered to nonfasted Jcl: C57BL/6N mice (male, 8 weeks old, $n = 3$) orally (3 mg/kg, 0.5% methylcellulose suspension). At 15 and 30 min and 1, 2, 4, 8, 24 h after oral administration, blood and brain samples were collected. The blood samples were centrifuged to obtain the plasma fraction. The brain samples were homogenized in saline to obtain the brain homogenate. The plasma and brain homogenate samples were deproteinized with acetonitrile containing an internal standard. After centrifugation, the supernatant obtained was diluted with 0.01 mol/L ammonium acetate and centrifuged again. The compound concentration in the supernatant was measured by LC/MS/MS with an API4000 triple quadrupole mass spectrometer (MDS Sciex). The mass spectrometer was equipped with a turbo ionspray source and operated in positive ion mode. The HPLC conditions were as follows: column, Chiralcel OJ-RH (2.1 mm × 150 mm); mobile phase, (A) 0.01 mol/L ammonium acetate/(B) acetonitrile = 6/4; gradient condition, 0−7 min: 40% B; 7−7.2 min: 40−85% B; 7.2−13.5 min: 85% B; 13.5−14 min: 85−40% B; 14−30 min: 40% B; flow rate, 0.2 mL/min; column temperature, 40 °C.

Bioavailability Analysis. Test compounds were administered as a cassette dosing to nonfasted rats. After oral and intravenous administration, blood samples were collected. The blood samples were centrifuged to obtain the plasma fraction. The plasma samples were deproteinized with acetonitrile containing an internal standard. After centrifugation, the supernatant was diluted with 0.01 mol/L ammonium acetate and centrifuged again. The compound concentrations in the supernatant were measured by LC/MS/MS.

Solubility Determination. Small volumes of the compound DMSO solutions were added to the aqueous buffer (pH 6.8). After incubation, precipitates were separated from by filtration through a filter plate. The filtrates were analyzed for compound in solution by HPLC analysis.

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Supporting Information Available: Elemental analysis data. This material is available free of charge via the Internet at http://pubs.acs.org.

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